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Updated Search IBF 4/18/05

DATE: Monday, April 18, 2005

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☐	L1	neospora.ti,ab,clm.	22
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☐	L2	neospora.ti,ab,clm.	119
☐	L3	L2 not l1	97
☐	L4	l3 and temperature	21

END OF SEARCH HISTORY

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☐	L1	neospora.ti,ab,clm.	22

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☐ 1. 6787146. 09 Nov 01; 07 Sep 04. Neospora vaccine. Brake; David A., et al. 424/269.1; 424/184.1 424/193.1 424/265.1. A61K039/002.

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☐ 1. Document ID: US 20050003433 A1

Using default format because multiple data bases are involved.

L4: Entry 1 of 21 File: PGPB Jan 6, 2005

PGPUB-DOCUMENT-NUMBER: 20050003433
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050003433 A1

TITLE: Recombinant neospora antigens and their uses

PUBLICATION-DATE: January 6, 2005

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/69.3, 435/7.22, 530/350, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 2. Document ID: US 20040223982 A1

L4: Entry 2 of 21 File: PGPB Nov 11, 2004

DOCUMENT-IDENTIFIER: US 20040223982 A1
TITLE: Neospora vaccine

Abstract Paragraph:
The present invention provides an homogenate prepared from cells of Neospora, and vaccines against neosporosis prepared therefrom which are useful in the prevention of clinical disease and abortion in mammals.

Detail Description Paragraph:
[0036] Cells which may be used to produce the cell homogenate of the invention are preferably tachyzoites, but may alternatively be bradyzoites or oocysts, or some combination thereof. In addition, cells for use in the present invention may either be viable cells or cells which have previously been inactivated, e.g., by treatment with a chemical inactivating agents such as formaldehyde or glutaraldehyde, among

others, or by treatment with radiation, or by exposure to extreme pH or temperature, or some combination thereof.

Detail Description Paragraph:
[0069] The tachyzoite preparation was frozen (-20.degree. C.) and thawed (room temperature) three times, and then sonicated (Branson Sonifer 250, Branson Inc.) at a constant output (4 minutes/cycle) for three cycles on ice. The resulting homogenate was designated as a Neospora antigen (NSA) preparation. The protein concentration of the NSA preparation was determined using a commercial assay (Pierce BCA). NSA preparation aliquots were prepared and stored at -20.degree. C. or -70.degree. C. until further use in a vaccine and for in vitro assays (e.g., Western blot, cell proliferation). The NSA preparation did not contain any viable tachyzoites, as determined by lack of in vitro growth in MARC-145 cells and the inability to kill immunodeficient, nude mice.

Detail Description Paragraph:
[0074] In the first part of the study, 8 week old female BALB/c mice (n=10/group) were immunized at day 0 and again at day 21 with either the SEAM62 adjuvant alone (control) or the NSA preparation plus the SEAM62 adjuvant (vaccine). Fifteen days after the last immunization, individual serum samples were randomly collected from 3 mice per group and stored at -20.degree. C. for analysis of parasite-specific antibodies by Western blot (FIG. 1). The post-immunization Western blot analysis was conducted as follows. The NSA preparation was fractionated alongside molecular weight markers (Novex, San Diego, Calif.) under standard, nonreducing conditions by preparative gel electrophoresis (SDS-PAGE) using 12% sodium dodecyl sulfate-polyacrylamide precast gels (Novex). Following electrophoresis, separated proteins were transferred to PVDF membrane (Millipore, Bedford, Mass.), which was then rinsed in wash buffer (phosphate buffered saline (pH 7.5)/0.5% Tween 20 detergent), air-dried, and individual membrane strips cut (approx. 8 .mu.g NSA protein/strip). Strips were incubated overnight at room temp. in blocking buffer (wash buffer containing 5% skim milk). Following two brief washes, strips were incubated for 1 hr at room temp. with primary antiserum samples (1:200 dilution in wash buffer) obtained at 15 days after the last immunization from either 3 individual adjuvant control mice (FIG. 1, lanes 1-3) or 3 vaccinated mice (FIG. 1, lanes 4-6). Following two rinses in wash buffer, strips were incubated with alkaline-phosphatase conjugated goat anti-mouse IgG (Kirkegaard & Perry) (1:10,000 dilution in wash buffer) for 1 hr at room temp, rinsed twice in wash buffer, and immunoreactive proteins detected using the chromogenic substrate BCIP/NBT (5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium) (Kirkegaard & Perry).

Detail Description Paragraph:
[0078] Pre- and post-challenge immunofluorescence antibody (IFA) titer assays were conducted as follows. Viable NC-1 tachyzoites (5.times.10.sup.4) were added to each well of a 96-well flat bottom plate. Wells were air-dried, and plates were stored at -20.degree. C. until used. Serum test samples collected on day 21 post-immunization (day 0 challenge) and day 21 post-challenge were tested for IFA titers. Starting at an initial 1:50 serum dilution, serial twofold dilutions were added to wells and incubated for 30 min at room temperature. Following two washes in carbonate rinse buffer, wells were incubated with (Fab).sub.2 fluorescein isothiocyanate-conjugated anti-mouse IgG+IgM (Southern Biotechnology, Birmingham., Ala.) The plates were washed and 50 .mu.l of 50% glycerol diluted in rinse buffer was added to each well. Plates were stored at 4.degree. C. until viewed under a fluorescence microscope equipped with a filter for emission at 510 nm. Antibody titers are based on the highest dilution of immune serum producing detectable fluorescence.

CLAIMS:

1. An homogenate prepared from cells of Neospora which is capable of inducing a protective response against neosporosis in a mammal.

2. The homogenate of claim i., wherein the species of Neospora from which the homogenate is prepared is N. caninum.

4. The homogenate of claim 3, wherein the species of Neospora from which the homogenate is prepared is N. caninum.

7. A vaccine to protect a mammal against neosporosis, comprising an immunologically effective amount of an homogenate prepared from cells of Neospora, which homogenate is capable of inducing a protective response against neosporosis in a mammal, and a veterinarily acceptable carrier.

8. The vaccine of claim 7, wherein the species of Neospora from which the homogenate is prepared is N. caninum.

10. The vaccine of claim 9, wherein the species of Neospora from which the homogenate of the vaccine is prepared is N. caninum.

18. The vaccine of claim 7, wherein the Neospora cells from which the homogenate is prepared have been modified to delete the expression of one or more antigenic components normally associated with Neospora cells or a homogenate prepared therefrom.

19-38. (Canceled).

39. A combination vaccine for protecting a mammal against neosporosis and, optionally, one or more other diseases or pathological conditions that can afflict the mammal, which combination vaccine comprises an immunologically effective amount of a first composition comprising an homogenate prepared from cells of Neospora, which homogenate is capable of inducing a protective response against neosporosis in a mammal; an immunologically effective amount of a second composition capable of inducing a protective response against a disease or pathological condition that can afflict the mammal; and a veterinarily acceptable carrier.

40. The combination vaccine of claim 39, wherein the species of Neospora from which the homogenate of the first composition is prepared is N. caninum.

42. The combination vaccine of claim 41, wherein the species of Neospora from which the homogenate of the first composition is prepared is N. caninum.

45. The combination vaccine of claim 39, wherein the second composition is capable of inducing in the mammal a protective response against a pathogen selected from the group consisting of bovine herpes virus, bovine respiratory syncitial virus, bovine viral diarrhea virus, parainfluenza virus types I, II, or III, Leptospira spp., Campylobacter spp., Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma spp., Klebsiella spp., Salmonella spp., rotavirus, coronavirus, rabies, Pasteurella haemolytica, Pasteurella multocida, Clostridia spp., Tetanus toxoid, E. coli, Cryptosporidum spp., Eimeria spp. and Neospora spp.

46. A kit for vaccinating a mammal against neosporosis, comprising a first container having an immunologically effective amount of an homogenate prepared from cells of Neospora, which homogenate is capable of inducing a protective response against neosporosis in a mammal, and a second container having a veterinarily acceptable carrier or diluent.

47-51. (Canceled)

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

3. Document ID: US 20040141986 A1

L4: Entry 3 of 21 File: PGPB Jul 22, 2004

DOCUMENT-IDENTIFIER: US 20040141986 A1
TITLE: Multicomponent vaccine containing clostridial and non-clostridial organisms in a low dose

Summary of Invention Paragraph:
[0028] Clostridium chauvoei causes the disease blackleg. This organism, like all Clostridial organisms, produces spores that can survive in soil for years and, during this time, can infect susceptible animals (cattle and sheep) which ingest them. Blackleg is an acute, infectious but noncontagious, disease of cattle and sheep characterized by gaseous tissue swelling, usually in the heavy muscles. The organism enters cattle or sheep via feed or cuts or by shearing, docking, or castration. The onset of the disease is quite sudden. Body temperature rises rapidly and muscular stiffness, depression and reluctance to move are prominent. When infection is extensive, death often occurs within 16-72 hours. Treatment of sick animals is futile since there is often permanent damage done to their meat.

Summary of Invention Paragraph:
[0040] Infectious bovine rhinotracheitis virus causes a severe respiratory infection of cattle, specifically in feedlot conditions. The disease is characterized by: high temperature, excessive nasal discharge, conjunctivitis and ocular discharge, inflamed nasal mucosa, increased rate of respiration, coughing, loss of appetite, depression and/or reproductive failure in cattle. Infection with this virus often predisposes cattle to bacterial infections that cause death.

Summary of Invention Paragraph:
[0041] Parainfluenza type 3 virus (PI.sub.3) usually causes a localized upper respiratory tract infection, producing elevated temperatures and moderate nasal and ocular discharge. Although clinical signs of PI.sub.3 are typically mild, this infection weakens the respiratory defenses and allows replication of other pathogens, particularly Pasteurella spa.

Summary of Invention Paragraph:
[0042] Bovine virus diarrhea (BVD) is a major cause of abortion, fetal resorption or congenital fetal malformation. If susceptible cows are infected with non cytopathic BVD virus during the first trimester of pregnancy, their calves may be born persistently infected with the virus. Exposure of those calves to certain virulent cytopathic BVD virus strains may precipitate BVD-mucosal disease. Clinical signs of this disease include loss of appetite, ulcerations in the mouth, profuse salivation, elevated temperature, diarrhea, dehydration and lameness. The disease usually affects feedlot cattle.

CLAIMS:

9. The vaccine according to claim 4 wherein the parasite is selected from th group consisting of Neospora spp., Tritrichimonas foetus and Cryptosporidium bovis.

31. The vaccine according to claim 28 wherein the parasite is selected from the group consisting of Neospora spp., Tritrichimonas foetus and Cryptosporidia spp.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

4. Document ID: US 20040081666 A1

L4: Entry 4 of 21 File: PGPB Apr 29, 2004

DOCUMENT-IDENTIFIER: US 20040081666 A1
TITLE: Cattle reproductive disease vaccines

Summary of Invention Paragraph:
[0005] BVDV is classified in the pestivirus genus and Flaviviridae family. It is closely related to viruses causing border disease in sheep and classical swine fever. Infected cattle exhibit "mucosal disease" which is characterized by elevated temperature, diarrhea, coughing and ulcerations of the alimentary mucosa (Olafson, et al., Cornell Vet. 36:205-213 (1946); Ramsey, et al., North Am. Vet. 34:629-633 (1953)). The BVD virus is capable of crossing the placenta of pregnant cattle and may result in the birth of PI calves (Malmquist, J. Am. Vet. Med. Assoc. 152:763-768 (1968); Ross, et al., J. Am. Vet. Med. Assoc. 188:618-619 (1986)). These calves are immunotolerant to the virus and persistently viremic for the rest of their lives. They provide a source for outbreaks of mucosal disease (Liess, et al., Dtsch. Tieraerztl. Wschr. 81:481-487 (1974) and are highly predisposed to infection with microorganisms causing diseases such as pneumonia or enteric disease (Barber, et al., Vet. Rec. 117:459-464 (1985).

Summary of Invention Paragraph:
[0009] Modified-live virus (MLV) vaccines, on the other hand, offer a higher level of protection. Currently, licensed BVDV MLV vaccines are produced using attenuated viruses obtained via repeated passage in bovine or porcine cells (Coggins et al., Cornell Vet. 51: 539-, 1961; Phillips et al., Am. J. Vet. Res. 36: 135-, 1975), or using chemically modifiedviruses which exhibit a temperature-sensitive phenotype (Lobmann et al., Am. J. Vet. Res. 45: 2498-, 1984; 47: 557-561, 1986). A single dose of MLV vaccine is sufficient for immunization, and duration of the immunity can last for years in vaccinated cattle. However, as these vaccines have been developed using type I BVDV virus strains, the protection is against type I virus only. Moreover, the available BVDV vaccines are not indicated for use in pregnant cattle or calves nursing pregnant cows.

Summary of Invention Paragraph:
[0020] The term "treating or preventing" with respect to a disease or disorder as used herein means reducing or eliminating the risk of infection by a virulent BVDV virus, types I and 2; IBR; P13; BRSV; Campylobacteria; and/or Leptospira antigens, ameliorating or alleviating the symptoms of an infection, or accelerating the recovery from an infection. The treatment is considered therapeutic if there is a reduction in viral or bacterial load, decrease in pulmonary infections, reduced rectal temperatures, and/or increase in food uptake and/or growth. The treatment is also considered therapeutic if there is a reduction in fetal infection and urinary shedding due to infection with Leptospira serovars hardjo and pomona, for example.

Summary of Invention Paragraph:
[0021] The method of the present invention is, for example, effective in preventing or reducing abortion caused by IBR and infections caused by BVDV Types 1 and 2, and reducing rectal temperatures. The present invention is therefore contemplated to provide fetal protection against IBR and infections caused by BVDV Types 1 and 2 as well as fetal protection against cattle herpes and cattle pestiviruses. The present invention is also contemplated to provide protection against persistent fetal

infection, such as persistent BVDV infection. By "persistent fetal infection" is meant infection occurring during early fetal development (e.g., 45-125 days of gestation) that leads to the live birth of animals that are immunotolerant to BVDV and maintain active BVDV replication and multiplication that often occurs at a high rate for months or years, serving as a permanent source of BVDV in the herd. These persistently infected animals are also at risk of developing fatal mucosal disease if superinfected with a cytopathic virus biotype.

Summary of Invention Paragraph:
[0022] The term "combination vaccine" is meant a bivalent or multivalent combination of antigens including modified live antigens and/or inactivated antigens. In accordance with the present invention a combination vaccine can comprise modified live infectious IBR, PI3, BRSV and inactivated BVDV Types 1 and 2, one or more antigens such as but not limited to Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii hardio-prajitno, Leptospira icterohaemmorrhagia, Leptospira interrogans pomona, Leptospira borgpetersenii hardjo-bovis, Leptospira bratislava, Campylobacter fetus, Neospora caninum, Trichomonus fetus, Mycoplasma bovis, Haemophilus somnus, Mannheimia haemolytica and Pasturella multocida, a veterinary acceptable carrier and an adjuvant. In a preferred embodiment the modified live IBR component is temperature sensitive IBR. In another preferred embodiment the BVDV Type 2 component is cytopathic (cpBVD-2 strain 53637-ATCC No. PTA-4859) and the BVDV Type 1 component is cytopathic 5960 (cpBDV-1 strain 5960-National Animal Disease Center, United States Department of Agriculture, Ames, Iowa). The present invention also contemplates non-cytopathic BVDV Type 1 and Type 2 strains. In still another preferred embodiment, the modified live antigens are desiccated, lyophilized or vitrified.

Detail Description Paragraph:
[0123] Two groups of 16 cattle were vaccinated twice subcutaneously at an interval of 3 weeks with 2 mL of L. hardjo/L. pomona combination vaccines prepared from two adjuvant formulations: 1) 2.5% Amphigen with Quil A /cholesterol each at 250 mcg/mL, and 2) Amphigen/AI-gel. The vaccines consisted of killed leptospires from which the culture fluids had been removed, so free endotoxin was low. Body temperatures, injection-site reactions, and general health observations were recorded following both injections. No systemic affects were seen, and local reactions were minimal and judged to be clinically acceptable. Sixteen additional cattle were injected with saline as controls. Four weeks after vaccination, cattle were challenged by ocular and vaginal instillation of 5 x 10.sup.6 leptospires on 3 consecutive days. Half of each treatment group was challenged with serovar hardjo and half with pomona. Two pomona controls were eliminated from the study for unrelated reasons, leaving 6 animals in that group. Urine collected weekly, and kidney samples collected at necropsy, 8 weeks after challenge, were evaluated by culture, PCR, and fluorescent antibody microscopy (FA).

CLAIMS:

18. A method of inducing an immune response against an antigen selected from the group consisting of Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii hardio-prajitno, Leptospira icterohaemmorrhagia, Leptospira interrogans pomona, Leptospira borgpetersenii hardjo-bovis, Leptospira bratislava, Neospora caninum, Trichomonus fetus, Mycoplasma, bovis, Haemophilus somnus, Mannheimia haemolytica and Pasturella multocida in an animal subject, comprising administering an immunologically effective amount of the composition of claim 1 and a veterinary-acceptable carrier.

54. A method of treating or preventing a disease or disorder in an animal caused by infection with an antigen selected from the group consisting Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii hardio-prajitno, Leptospira icterohaemmorrhagia, Leptospira interrogans pomona, Leptospira borgpetersenii hardjo-bovis, Leptospira Bratislava, Campylobacter fetus, Neospora caninum,

Trichomonus fetus, Mycoplasma, bovis, Haemophilus somnus, Mannheimia haemolytica and Pasturella multocida, comprising administering to said animal a therapeutically effective amount of the vaccine composition of claim 20.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

5. Document ID: US 20040062772 A1

L4: Entry 5 of 21 File: PGPB Apr 1, 2004

DOCUMENT-IDENTIFIER: US 20040062772 A1
TITLE: Attenuated live neospora vaccine

Abstract Paragraph:

The present invention provides attenuated live cultures of the pathogenic protozoan parasite, Neospora, and live vaccines against neosporosis prepared therefrom which are useful in the prevention of clinical disease and abortion in mammals.

Summary of Invention Paragraph:

[0020] High serial passage may be carried out by repeated in vitro passaging of cells of a pathogenic strain of Neospora in susceptible host cells until sufficient attenuation occurs. Passaging may be conducted under specific environmental conditions to select for attenuated cells. For example, passaging, may be conducted at a temperature below that of the body temperature of the intended mammalian vaccinate to select for temperature-sensitive strains of Neospora that will not grow, or that will only grow at a reduced rate, when administered in a vaccine to the mammal.

Summary of Invention Paragraph:

[0033] After the attenuation step, cells that exhibit one or more indicators of attenuated pathogenicity are selected from the culture and clonally propagated after limiting dilution. Examples-of such indicators include, but are not limited to, appearance of a novel temperature-sensitivity or a novel auxotrophy in vitro, or a reduction in a virulence attribute such as infectivity or severity or rate of progression of one or more symptoms or conditions in a mammal after administration of cells of the strain as compared to infection with the parent strain, among others. A particular, non-limiting example of a temperature-sensitivity that is useful in practicing the invention is on in which cells of the attenuated strain will grow at 32.degree. C., but not at 37.degree. C. Such a temperature-sensitive strain will cause the lysis of infected host cells at 32.degree. C., resulting in the appearance of lesions or plaques in a host cell monolayer. When grown at 37.degree. C., the attenuated strain will not replicate sufficiently and will thus fail to produce plaques in host cell monolayers.

Detail Description Paragraph:

Establishment Of Temperature-Sensitive Strains of N. caninum and Analysis of Pathogenicity in BALB/c Mice

Detail Description Paragraph:

[0050] The objective of this study was to establish temperature-sensitive strains of N. caninum (NCTS), and to test the pathogenicity of these strains by analyzing serum antibody response, tissue cyst and brain lesion production, and the development of clinical symptoms in BALB/c mice, which are known to be highly

susceptible to neosporosis.

Detail Description Paragraph:
[0052] Tachyzoites of the NC-1 strain were mutagenized by exposure to 0.5 .mu.M N-methyl-N'-nitro-N-nitrosoguanidine (Sigma) in growth medium for 24 hr, and then grown at 32.5.degree. C. for 3 mos in Hs68 cells in maintenance medium, after which tachyzoites were cloned by limiting dilution. Twelve clones were initially isolated. Three clones, designated as NCTS-4, NCTS-8, and NCTS-12 (NCTS=N. caninum, temperature-sensitive), were selected for further study after being maintained in Hs68 cells in continuous culture at 32.5.degree. C. for >8 mos in maintenance medium.

Detail Description Paragraph:
[0083] A first objective of this study was to determine if vaccination with a live, temperature-sensitive strain of N. caninum can provide protection against disease caused by subsequent challenge with a pathogenic strain, e.g., NC-1. of N. caninum. A second objective of this study was to determine the level of protection provided by vaccination of BALB/c mice with killed (frozen) NCTS8 tachyzoites that were subsequently challenged with the NC-1 strain of N. caninum.

Detail Description Paragraph:
[0112] Does in groups A-D were challenged with 4.times.10.sup.6 tachyzoites of-the NC-1 strain of N. caninum in serum-free maintenance medium (0.45 ml) administered by jugular i.v. The does were then monitored by ultrasound, by temperature taken daily for 7 days post-challenge, and by visual observation twice daily, and were bled once per week post-challenge.

CLAIMS:

1. A culture of cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects a mammal against neosporosis when administered as a live vaccine.

2. The culture of claim 1, the cells of which are temperature-sensitive.

6. A vaccine to protect a mammal against neosporosis, comprising an immunologically effective amount of live cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects the mammal against neosporosis when administered as a live vaccine, and a veterinarily acceptable carrier.

7. The vaccine of claim 6, wherein the attenuated cells are temperature-sensitive.

13. A method for preparing a culture of attenuated cells of a species of Neospora for use in a vaccine that protects a mammal against neosporosis, comprising modifying cells from a pathogenic parent strain of a species of Neospora; selecting and clonally propagating one or more modified cells that exhibit attenuated pathogenicity compared to cells of the parent strain; and selecting and clonally propagating one or more attenuated cells which are capable of triggering an immune response that protects the mammal against neosporosis when administered in a live vaccine.

14. The method of claim 13, in which the cells of the attenuated culture are temperature-sensitive.

17. A method for preparing a vaccine to protect a mammal against neosporosis, comprising modifying cells from a pathogenic parent strain of a species of Neospora; selecting and clonally propagating those modifi d cells that exhibit

attenuat d pathogenicity compared to cells of the parent strain but which are capable of triggering an immune response in the mammal that protects against neosporosis when administered in a live vaccine; and combining an immunologically effective amount of the attenuated cells with a veterinarily acceptable carrier in a form suitable for administration as a live vaccine to the mammal.

18. The method of claim 17, wherein the attenuated cells are temperature-sensitive.

24. A method of vaccinating a mammal against neosporosis, comprising administering to the mammal an immunologically effective amount of a vaccine comprising live cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects the mammal against neosporosis when administered as a live vaccine, and a veterinarily acceptable carrier.

25. The method of claim 24, wherein the attenuated cells are temperature-sensitive.

32. A combination vaccine, comprising an immunologically effective amount of live cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects the mammal against neosporosis when administered as a live vaccine; one or more other antigens that trigger an immune response that protects the mammal against a disease or a pathological condition; and a veterinarily acceptable carrier.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

6. Document ID: US 20030219381 A1

L4: Entry 6 of 21 File: PGPB Nov 27, 2003

DOCUMENT-IDENTIFIER: US 20030219381 A1
TITLE: Animal model for infection by an apicomplexan parasite

Detail Description Paragraph:
[0109] S. neurona was maintained as previously described in EXAMPLE 1. Parasites were freshly harvested and used to infect homologous cells obtained from the hosts (horse) peripheral blood. The host cells were concentrated to obtain an enriched fraction of white blood cells (lymphocytes). Culture derived merozoites grown and maintained in bovine turbinate (BT) cells as previously described were removed from a flask that contained free merozoites in the media and centrifuged at 600.times.G G for 10 minutes at room temperature. The merozoites were resuspended in RPMI growth media and counted using a hemocytometer. Twenty four thousand equine purified lymphocytes obtained from the peripheral blood were added to each of six microfuge tubes and twenty four thousand merozoites were added to each tube. The tubes were incubated for 2 to 24 hours 37.degree. C. in 5% CO.sub.2. The tubes were under layered with density 1.077 Optiprep and 250 .mu.l of phosphate buffered saline (PBS) was over layered on the gradient. The microfuge tube containing the gradient was centrifuged at 200.times.G for twenty minutes at room temperature. The supernate above the interface was collected and diluted with an equal volume of RPMI medium and centrifuged at 5000.times.G for five minutes. The number of viable parasites in homologous lymphocytes was determined by counting 5 .mu.l of infected

cells dried on a slide and stained with Geimsa. It was determined that the parasites had entered cells at 5 hours and the infected cells were added back to a 6 ml EDTA tube containing fresh peripheral blood from the homologous horse. An example of such an infected B lymphocyte of a horse is shown in the photograph in FIG. 2.

CLAIMS:

2. The method of claim 1 wherein the Apicomplexan parasite is selected from the group consisting of Sarcocystis. dasypus (syn. S. neurona), Sarcocystis. neurona, Sarcocystis falcatula, Toxoplasma. gondii, Neospora. caninum, Neospora. hughesi, Sarcocystis cruzi, Sarcocystis sp., Eimeria sp. and Plasmodium sp.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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7. Document ID: US 20030185852 A1

L4: Entry 7 of 21 File: PGPB Oct 2, 2003

DOCUMENT-IDENTIFIER: US 20030185852 A1
TITLE: Parasitic protozoan isolate

Abstract Paragraph:
The present invention relates to a novel Neospora caninum isolate from Nowra and extracts thereof. The strain is useful in the development of diagnostic assays for the detection of parasites in animals. The present invention also relates to pharmaceutical compositions, using live or killed organisms or extracts thereof, for the treatment and prevention of parasitic infections in animals.

Summary of Invention Paragraph:
[0012] The literature on live vaccines against N. caninum is limited. Atkinson et al. (1999) showed that infection of naive mice by the Nc-SweB1 isolate of N. caninum partially protected them against a severe infection by Nc-Liverpool. Lindsay et al. (1999) generated temperature sensitive mutants of N. caninum and demonstrated that they could prevent clinical signs associated with neosporosis in mice.

Detail Description Paragraph:
[0055] After formulation, the vaccine may be incorporated into a sterile container which is then sealed and stored at a low temperature, for example 4.degree. C., or it may be freeze-dried. Lyophilisation permits long-term storage in a stabilised form.

Detail Description Paragraph:
[0076] Cell proliferation assays were performed on mouse spleen cells as follows. Spleens were individually placed in wash medium (DMEM containing penicillin and streptomycin, DMEM(PS), and forced through a 70 .mu.m nylon disposable sieve to yield a single cell suspension. The cells were concentrated by centrifugation and resuspended in 2 mls red cell lysis buffer and left at room temp. for 1 min. The lysis buffer was diluted out with more DMEM/PS, centrifuged and the pellets resuspended in fresh DMEM/PS for counting. 1.times.10.sup.4 cells per well of each suspension were added in triplicate to wells of a microtitre plate. Stimulating antigen at a concentration of 10 .mu.g/well for Con A and Nc-Nowra lysate was added

to the cells. A blank (containing no cells) was included. The cells were incubated at 37.degree. C. in 5% CO.sub.2 for 4 days. BrdU was then added and the cells incubated for a further 4 hours. The cells were pelleted to the bottom of the microtitre plate by centrifugation, the supernatants collected for cytokine assay, and the cells dried and fixed. BrdU incorporation was then detected using a commercial immunoassay kit (Roche) encompassing an anti-BrdU-POD conjugate.

Detail Description Paragraph:
[0119] Lindsay D S, Lenz S D, Blagburn B L & Brake D A (1999) Characterization of temperature-sensitive strains of Neospora caninum in mice Journal of Parasitology 85, 64-67.

CLAIMS:

14. A method as claimed in any one of claims 10 to 13 wherein the infection or disease is caused by the presence of Neospora in the animal.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

8. Document ID: US 20030180785 A1

L4: Entry 8 of 21 File: PGPB Sep 25, 2003

DOCUMENT-IDENTIFIER: US 20030180785 A1
TITLE: Polynucleotide molecules encoding Neospora proteins

Abstract Paragraph:
The present invention provides isolated polynucleotide molecules comprising nucleotide sequences encoding GRA1, GRA2, SAG1, MIC1 and MAG1 proteins from Neospora caninum, as well as recombinant vectors, transformed host cells, and recombinantly-expressed proteins. The present invention further provides a polynucleotide molecule comprising the nucleotide sequence of the bidirectional GRA1/MAG1 promoter of N. caninum. The present invention further provides genetic constructs based on the polynucleotide molecules of the present invention that are useful in preparing modified strains of Neospora cells for use in vaccines against neosporosis.

Summary of Invention Paragraph:
[0097] Once a polynucleotide molecule of the present invention has been stably introduced into an appropriate host cell, the transformed host cell is clonally propagated, and the resulting cells are grown under conditions conducive to the maximum production of the encoded polypeptide. Such conditions typically include growing transformed cells to high density. Where the expression vector comprises an inducible promoter, appropriate induction conditions such as, e.g., temperature shift, exhaustion of nutrients, addition of gratuitous inducers (e.g., analogs of carbohydrates, such as isopropyl-.beta.-D-thiogalactopyranosid- e (IPTG)), accumulation of excess metabolic by-products, or the like, are employed as needed to induce expression.

CLAIMS:

1. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a Neospora GRA1 protein, said nucleotide sequence selected from the group consisting

of the nucleotide sequence of the open reading frame (ORF) of SEQ ID NO: 1 from about nt 205 to about nt 777; the nucleotide sequence of the ORF of SEQ ID NO: 3 from about nt 605 to about nt 1304; the nucleotide sequence of the GRA1-encoding ORF of plasmid pRC77 (ATCC 209685); and a nucleotide sequence that is homologous to any of the aforementioned nucleotide sequences.

6. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a Neospora GRA2 protein, said nucleotide sequence selected from the group consisting of the nucleotide sequence of the ORF of SEQ ID NO: 4 from about nt 25 to about nt 660; the nucleotide sequence of the GRA2-encoding ORF of plasmid pRC5 (ATCC 209686); and a nucleotide sequence that is homologous to any of the aforementioned nucleotide sequences.

11. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a Neospora SAG1 protein, said nucleotide sequence selected from the group consisting of the nucleotide sequence of the ORF of SEQ ID NO: 6 from about nt 130 to about nt 1089; the nucleotide sequence of the SAG1-encoding ORF of plasmid pRC102 (ATCC 209687); and a nucleotide sequence that is homologous to any of the aforementioned nucleotide sequences.

16. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a Neospora MIC1 protein, said nucleotide sequence selected from the group consisting of the nucleotide sequence of the ORF of SEQ ID NO: 8 from about nt 138 to about nt 1520; the nucleotide sequence of the ORF of SEQ ID NO: 10; the nucleotide sequence of the MIC1-encoding ORF of plasmid pRC340 (ATCC 209688); and a nucleotide sequence that is homologous to any of the aforementioned nucleotide sequences.

21. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a Neospora MAG1 protein, said nucleotide sequence selected from the group consisting of the nucleotide sequence of the ORF of SEQ ID NO: 11 from about nt 1305 to about nt 2786; the nucleotide sequence of the ORF of SEQ ID NO: 12 from about nt 122 to about nt 1381 the nucleotide sequence of the MAG1-encoding ORF of plasmid bd304 (ATCC 203413); and a nucleotide sequence that is homologous to any of the aforementioned nucleotide sequences.

35. A genetic construct comprising a polynucleotide molecule that can be used to disable a Neospora gene, comprising: (a) a polynucleotide molecule comprising a nucleotide sequence that is otherwise the same as a nucleotide sequence of a GRA1, GRA2, SAG1, MIC1 or MAG1 gene from N. caninum, or that is otherwise the same as a nucleotide sequence that is homologous thereto, or a substantial portion of said nucleotide sequence, but which nucleotide sequence further comprises one or more mutations capable of disabling the GRA1, GRA2, SAG1, MIC1 or MAG1 gene from N. caninum; or (b) a polynucleotide molecule comprising a nucleotide sequence that naturally flanks in situ the ORF of a Neospora GRA1, GRA2, SAG1, MIC1, or MAG1 gene, or a nucleotide sequence that is homologous to said flanking sequence; such that transformation of a Neospora cell with the genetic construct of (a) or (b) results in disabling of the GRA1, GRA2, SAG 1, MIC1 or MAG1 gene.

38. A Neospora cell that has been modified by transformation with the genetic construct of claim 35 such that the GRA1, GRA2, SAG1, MIC1 or MAG1 gene, or a combination of said genes, has been disabled.

39. A method of preparing modified Neospora cells, comprising transforming Neospora cells with the genetic construct of claim 35, and selecting transformed cells that express a mutant phenotype selected from the group consisting of GRA1.sup.-, GRA2.sup.-, SAG.sup.-, MIC1.sup.-, and MAG.sup.- as a result of said transformation.

40. A vaccine against neosporosis, comprising an immunologically effective amount of a component comprising: (a) a polypeptide selected from the group consisting of:

(i) an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein; (ii) a polypeptide having an amino acid sequence that is homologous to an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein; (iii) a polypeptide consisting of a substantial portion of an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein, or polypeptide which is homologous thereto; (iv) a fusion protein comprising the protein or polypeptide of (i), (ii) or (iii); and (v) an analog or derivative of the protein or polypeptide of (i), (ii), (iii) or (iv); (b) a polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide of (a); or (c) a Neospora cell that has been modified by transformation with the genetic construct of claim 35 such that the GRA1, GRA2, SAG1, MIC1 or MAG1 gene, or a combination of said genes, in said cell has been disabled; and a veterinarily acceptable carrier.

41. The vaccine of claim 40, wherein the modified Neospora cells are live cells.

42. The vaccine of claim 40, wherein the modified Neospora cells are inactivated cells.

47. A method of preparing a vaccine against neosporosis, comprising combining an immunologically effective amount of: (a) a polypeptide selected from the group consisting of: (i) an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein; (ii) a polypeptide having an amino acid sequence that is homologous to an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein; (iii) a polypeptide consisting of a substantial portion of an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein, or polypeptide which is homologous thereto; (iv) a fusion protein comprising the protein or polypeptide of (i), (ii) or (iii); and (v) an analog or derivative of the protein or polypeptide of (i), (ii), (iii) or (iv); (b) a polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide of (a); or (c) a Neospora cell that has been modified by transformation with the genetic construct of claim 35 such that the GRA1, GRA2, SAG1, MIC1 or MAG1 gene, or a combination of said genes, in said cell has been disabled; with a veterinarily acceptable carrier.

49. A kit for vaccinating a mammal against neosporosis, comprising a container comprising an immunologically effective amount of: (a) a polypeptide selected from the group consisting of: (i) an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein; (ii) a polypeptide having an amino acid sequence that is homologous to an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein; (iii) a polypeptide consisting of a substantial portion of an N. caninum GRA1, GRA2, SAG1, MIC1 or MAG1 protein, or polypeptide which is homologous thereto; (iv) a fusion protein comprising the protein or polypeptide of (i), (ii) or (iii); or (v) an analog or derivative of the protein or polypeptide of (i), (ii), (iii) or (iv); (b) a polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide of (a); or (c) Neospora cells that have been modified by transformation with the genetic construct of claim 35 such that the GRA1, GRA2, SAG1, MIC1 or MAG1 gene, or a combination of said genes, in said cells has been disabled.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KMC | Draw De

9. Document ID: US 20030166266 A1

L4: Entry 9 of 21 File: PGPB Sep 4, 2003

DOCUMENT-IDENTIFIER: US 20030166266 A1
TITLE: Open reading frame detection compositions and methods

Detail Description Paragraph:
[0071] If the target-gene(s) sequence:primer complex has been formed, the polymerase will cause the primers to be extended along the target-gene(s) sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target-gene(s) to form reaction products, excess primers will bind to the target-gene(s) and to the reaction products and the process is repeated. These multiple rounds of amplification, referred to as "cycles", are conducted until a sufficient amount of amplification product is produced.

Detail Description Paragraph:
[0075] Another method for amplification is the ligase chain reaction ("LCR"), disclosed in European Patent Application No. 320,308, incorporated herein by reference. In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR.TM., bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Pat. No. 4,883,750, incorporated herein by reference, describes a method similar to LCR for binding probe pairs to a target sequence.

Detail Description Paragraph:
[0211] The use of Taq DNA polymerase and mutants thereof is a more recent addition to the improvements of the Sanger method (U.S. Pat. No. 5,075,216). Taq polymerase is a thermostable enzyme that works efficiently at 70-75.degree. C. The ability to catalyze DNA synthesis at elevated temperature makes Taq polymerase useful for sequencing templates which have extensive secondary structures at 37.degree. C. (the standard temperature used for Klenow and Sequenase.TM. reactions). Taq polymerase, like Sequenase.TM., has a high degree of processivity and like Sequenase 2.0, it lacks 3' to 5' nuclease activity. The thermal stability of Taq and related enzymes (such as Tth and Thermosequenase.TM.) provides an advantage over T7 polymerase (and all mutants thereof) in that these thermally stable enzymes can be used for cycle sequencing, which amplifies the DNA during the sequencing reaction, thus allowing sequencing to be performed on smaller amounts of DNA. Optimization of the use of Taq in the standard Sanger Method has focused on modifying Taq to eliminate the intrinsic 5'-3' exonuclease activity and to increase its ability to incorporate ddNTPs to reduce incorrect termination due to secondary structure in the single-stranded template DNA (EP 0 655 506 B1). The introduction of fluorescently-labeled nucleotides has further allowed the introduction of automated sequencing, which further increases processivity.

Detail Description Paragraph:
[0294] The suitable conditions also mean that the incubation is at a temperature and for a period of time sufficient to allow effective binding. Incubation steps are typically from about 1 to 2 to 4 hours, at temperatures preferably on the order of 25.degree. to 27.degree. C., or may be overnight at about 4.degree. C. or so.

Detail Description Paragraph:
[0296] To provide a detecting means, the second or third antibody will have an associated label to allow detection. Preferably, this will be an enzyme that will generate color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one will desire to contact and incubate the first or second immune complex with a urease, glucose oxidase, alkaline phosphatase, or hydrogen peroxidase-conjugated antibody for a period of time and under conditions that favor the development of further immune complex formation, e.g., incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween.

Detail Description Paragraph:

[0336] The following information may also be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

CLAIMS:

9. The ORF selection vector of claim 7, wherein the parasite is Neospora caninum.

36. The method of claim 34, wherein the parasite is Neospora caninum.

57. The ORF selection vector of claim 55, wherein the parasite is Neospora caninum.

77. The method of claim 75, wherein the parasite is Neospora caninum.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw. De

10. Document ID: US 20030091591 A1

L4: Entry 10 of 21 File: PGPB May 15, 2003

DOCUMENT-IDENTIFIER: US 20030091591 A1
TITLE: Alphavirus expression vectors and uses thereof

Detail Description Paragraph:
[0066] Sindbis virus gene expression, which occurs in the cytoplasm of the cell, is quite efficient, rapid, and can be modulated. For example, Xiong et al., ibid., reported the production of up to 1.times.10.sup.8 molecules of chloramphenicol acetyltransferase (CAT) per cell transfected with Sindbis virus expression vectors operatively linked to the CAT gene, when the cell was cultured for about 20 hr. Xiong et al. also reported that use of a replication temperature sensitive Sindbis virus vector led to modulated expression of CAT.

Detail Description Paragraph:
[0081] After transfection, transfected cells are cultured in an effective medium, using techniques such as those described in Xiong et al., ibid. As used herein, an effective medium refers to any medium in which the transfected cells can produce recombinant virus particle vaccines. An effective medium is typically an aqueous medium comprising assimilable carbohydrate, nitrogen and phosphate sources, as well as appropriate salts, minerals, metals and other nutrients, such as vitamins, growth factors and hormones. Culturing is carried out at a temperature, pH and oxygen content appropriate for the transfected cell. , Such culturing conditions are well within the expertise of one of ordinary skill in the art. Examples of preferred effective media are included in the Examples section.

Detail Description Paragraph:
[0109] In order to produce protective compounds of the present invention, a recombinant cell, produced as described above, is cultured in an effective medium, using techniques such as those described in Xiong et al., ibid. As used herein, an effective medium refers to any medium in which the transfected cells can produce protective compounds of the present invention. An effective medium is typically an aqueous medium comprising assimilable carbohydrate, nitrogen and phosphate sources, as well as appropriate salts, minerals, metals and other nutrients, such as vitamins, growth factors and other hormones. The medium may comprise complex nutrients or may be a defined medium. Recombinant cells of the present invention can be cultured in conventional fermentation bioreactors, which include, but are not limited to, batch, fed-batch, cell recycle and continuous fermentors. Culturing can also be conducted in shake flasks, test tubes, microtiter dishes and petri plates. Culturing is carried out at a temperature, pH and oxygen content appropriate for the recombinant cell. Such culturing conditions are well within the expertise of one of ordinary skill in the art. Examples of preferred effective media and culturing conditions are included in the Examples section.

Detail Description Paragraph:
[0157] In one experiment, recombinant virus particle vaccine VPV SV2:nP30.1008 is produced by co-transfecting baby hamster kidney (BHK) cells with recombinant molecule SV2:nP30.1008 and packaging vector PV1 using electroporation in a manner similar to that described by Liljestrom et al., ibid. Briefly, BHK cells are grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells are trypsinized, washed once with Minimal Essential Medium (also called MEM; available from Life Technologies Inc., Gaithersburg, Md.) containing 10% fetal calf serum, washed once with ice cold phosphate buffered saline (8 g NaCl, 0.2 g KCl, 1.44 g Na.sub.2HPO.sub.4, 0.24 g KH.sub.2PO.sub.4 per liter of water, the pH of which is adjusted to about pH 7.4; also called PBS) and resuspended in PBS at about 1.times.10.sup.7 cells per ml. About 0.5 ml of cells and about 5-10 .mu.g (in about 10-50 microliters (.mu.l)) total of SV2:nP30.1008 and PV1 (at a mole/mole ratio of about 1:1) are mixed in a 0.2 centimeter (cm) cuvette suitable for use in Bio-Rad's Gene Pulser Apparatus (both available from Bio-Rad Laboratories, Richmond, Calif.). The RNA either may be used directly from the in vitro transcription reaction mixture (as described in Example 2 for the recombinant molecule and in Example 3 for the packaging vector) or may be diluted with transcription buffer containing 5 millimolar (mM) dithiothreitol and 1 unit of RNasin per ml. Electroporation is conducted at room temperature by two consecutive pulses at 1.5 kilovolts (KV) and 35 microfarads (.mu.F), using the Gene Pulser Apparatus with the pulse controller unit set at maximum resistance. After electroporation, the cells are diluted about 1:20 in complete BHK cell medium and transferred to tissue culture plates. The cells are then cultured for about 24 to about 36 hours at about 37.degree. C. and about 5% carbon dioxide in about 5 ml of MEM with 10% fetal calf serum.

Detail Description Paragraph:
[0167] A recombinant cell capable of expressing the GST-P 30.257 fusion protein was produced by transfecting SV3:nGST-nP30.771 into baby hamster kidney (BHK) cells using electroporation in a manner similar to that described by Liljestrom et al., ibid. Briefly, BHK cells were grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells were trypsinized, washed once with Minimal Essential Medium (also called MEM; available from Life Technologies Inc., Gaithersburg, Md.) containing 10% fetal calf serum, washed once with ice cold phosphate buffered saline (8 g NaCl, 0.2 g KCl, 1.44 g Na.sub.2HPO.sub.4, 0.24 g KH.sub.2P0.sub.4 per liter of water, the pH of which is adjusted to about pH 7.4; also called PBS) and resuspended in PBS at about 1.times.10.sup.7 cells per ml. About 0.5 ml of cells and about 5-10 .mu.g (in about 10-50 .mu.l) of SV3:nGST-nP30.771 were mixed in a 0.2 centimeter (cm) cuvette suitable for use in Bio-Rad's Gene Pulser Apparatus (both available from Bio-Rad Laboratories, Richmond, Calif.). The RNA was either used directly from the in vitro transcription reaction mixture (as described in Example 2) or was diluted with transcription buffer containing 5

millimolar (MM) dithiothreitol and 1 unit of RNasin per ml. Electroporation was conducted at room temperature by two consecutive pulses at 1.5 kilovolts (KV) and 35 microfarads (.mu.F), using the Gene Pulser Apparatus with the pulse controller unit set at maximum resistance. After electroporation, the cells were diluted about 1:20 in complete BHK cell medium and transferred to tissue culture plates.

Detail Description Paragraph:
[0181] A recombinant cell capable of expressing Di22.RA was produced by transfecting SV3:nDi22.RA into BHK cells using lipofection in a manner similar to that described by Feigner et al., pp. 7413-7417, 1987, Pxoc. Natl. Acad. Sci. USA, Vol. 84. Briefly, BHK cells were grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells were washed with PBS and incubated for about 10 minutes at about room temperature with a mixture of from about 0.25 to about 1.0 .mu.g of SV3:nDi22.RA and about 20 .mu.g of Lipofectin (available from Life Technologies Inc., Gaithersburg, Md.) in about 0.4 ml PBS. The mixtures was then removed and the cells washed two times with PBS and incubated with about 5 ml of MEM containing 10% fetal calf serum for about 24 to about 36 hours at about 37.degree. C. in order to produce recombinant virus. BHK cells were infected by the recombinant virus and incubated for about 12 to about 16 hours at about 37.degree. C. to produce Di22.RA.

Detail Description Paragraph:
[0184] Packaging-competent recombinant molecule SV3:nDi22.RA, produced as described in Example 9, was packaged into a viral coat as follows. SV3:nDi22.RA was transfected into BHK cells using lipofection in a manner similar to that described by Felgner et al., pp. 7413-7417, 1987, Proc. Natl. Acad. Sci. U.S.A., Vol. 84. Briefly, BHK cells were grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells were washed with PBS and incubated for about 10 minutes at about room temperature with a mixture of from about 0.25 to about 1.0 .mu.g of SV3:nDi22.RA and about 20 .mu.g of Lipofectin (available from Life Technologies Inc., Gaithersburg, Md.) in about 0.4 ml PBS. The mixtures was then removed and the cells washed two times with PBS and incubated with about 5 ml of MEM containing 10% fetal calf serum for about 24 to about 36 hours at about 37.degree. C. in order to produce recombinant virus RV SV3:nDi22.RA, also known as HJA.

CLAIMS:

5. The vaccine of claim 1, wherein said disease is caused by an infectious agent selected from the group consisting of the genera Toxoplasma, Dirofilaria, Acanthocheilonema, Babesia, Brugia, Candida, Cryptococcus, Cryptosporidium, Dipetalonema, Eimeria, Encephalitozoon, Hepatozoon, Histoplasma, Isospora, Loa, Microsporidia, Neospora, Nosema, Onchocerca, Parafilaria, Plasmodium, Pneumocystis, Rochalimaea, Setaria, Stephanofilaria, Theileria and Wuchereria.

42. The recombinant molecule of claim 40, wherein said parasite is selected from the group consisting of Toxoplasma, Dirofilaria, Acanthocheilonema, Babesia, Brugia, Candida, Cryptococcus, Cryptosporidium, Dipetalonema, Eimeria, Encephalitozoon, Hepatozoon, Histoplasma, Isospora, Loa, Microsporidia, Neospora, Nosema, Onchocerca, Parafilaria, Plasmodium, Pneumocystis, Rochalimaea, Setaria, Stephanofilaria, Theileria and Wuchereria.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

11. Document ID: US 20020165373 A1

L4: Entry 11 of 21 File: PGPB Nov 7, 2002

DOCUMENT-IDENTIFIER: US 20020165373 A1
TITLE: Recombinant neospora antigens and their uses

Abstract Paragraph:
The present invention provides isolated bovine Neospora cultures. Also provided are recombinant immunodominant Neospora antigens. The cultures and antigens are used to develop diagnostic assays for the detection of Neospora infections in cattle and other animals. Also provided are pharmaceutical compositions for the treatment and prevention of Neospora infections.

Detail Description Paragraph:
[0033] The phrase "selectively hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA. The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10.degree. C. lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30.degree. C. for short probes (e.g., 10 to 50 nucleotides) and at least about 60.degree. C. for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents as formamide.

Detail Description Paragraph:
[0092] An alternative means for detecting Neospora nucleic acids is in situ hybridization. In situ hybridization assays are well known and are generally described in Angerer, et al., Methods Enzymol., 152:649-660 (1987). In situ hybridization assays use cells or tissue fixed to a solid support, typically a glass slide. If DNA is to be probed, the cells are denatured with heat or alkali. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of labeled Neospora specific probes. The probes are preferably labeled with radioisotopes or fluorescent reporters.

Detail Description Paragraph:
[0111] Parasites in the tissue sections of brains from the 66th and 93rd fetus (hereafter referred to as fetus 66 and 93) were further characterized by the same immunohistochemical procedure to test their reactivity with antisera to additional apicomplexan protozoal parasites. Tissue sections were incubated at room temperature for 1 h with optimal dilutions of the following antisera: 1:1000 dilution of antiserum to N. caninum tachyzoites (Lindsay & Dubey, Am. J Vet. Res. 50:1981-3(1989)), 1:50 dilution of antiserum to Hainmondia hammondi tissue cysts

and 4 different antisera to T. gondii (Tg1-4). Antiserum Tg1 was produced by the infection of a rabbit with live sporulated oocysts of the ME-49 strain (Lindsay & Dubey, supra) of T. gondii and used at a 1:400 dilution. Toxoplasma gondii antiserum Tg2 (Dr J. C. Boothroyd, Stanford University) was produced by immunization of a rabbit with a tachyzoite lysate of the RH strain of T. gondii and was used at a dilution of 1:300. Antisera Tg3 (BioGenex Laboratories, Dublin, Calif., USA) and Tg4 (I.C.N. Immunobiologicals, Lisle, Ill., USA) were developed by immunizing rabbits with tachyzoites of the RH and H44 strains, respectively. Antiserum Tg3 was applied as supplied by the manufacturer and Tg4 was used at a 1:80 dilution. The optimal dilution chosen for each antiserum produced a strongly positive reaction against the respective positive control parasite with no appreciable non-specific, background staining. Control tissues consisted of paraffin-embedded sections of murine brain with N. caninum tachyzoites, murine brain with T. gondii cysts, murine spleen with T. gondii tachyzoites, murine skeletal muscle with H. hammondi cysts and bovine tongue with Sarcocystis cruzi cysts (Barr et al. Vet. Path. 28:110-1 16 (1991)).

Detail Description Paragraph:
[0134] Parasites were harvested for antigen preparation when approximately 80% of the CPAE cells in the culture flask were infected with clusters of tachyzoites. The infected monolayer was removed from the flask by scraping into the medium and then passed 3.times. through a 25 gauge needle to disrupt the cells. The suspension was passed through a 5 .mu.m filter to remove cellular debris and tachyzoites were pelleted by centrifugation at 1300.times.g for 10 min. After removing the supernatant, the pellet was washed twice in sterile phosphate buffered saline pH 7.2 (PBS) and then resuspended in a modified PBS saline (137 mM NaCl, 3 mM KCl, 3 mM Na.sub.3C.sub.6H.sub.5O.sub.7.2H.sub.2O, 0.4 mM NaH.sub.2PO.sub.4.H.sub.2O, 12 mM NaHCO.sub.3, 6 mM glucose) to a final concentration of approximately 2,000 tachyzoites/.mu.L. Aliquots of 10 .mu.L of tachyzoite suspension were dispensed into each 4 mm well on 12-well heavy teflon coated (HTC) antigen slides. Slides were air-dried at room temperature and stored at -70.degree. C.

Detail Description Paragraph:
[0140] Antigen slides were thawed at room temperature immediately prior to use. Sera were initially titrated in 2-fold dilutions from 1:40 to 1:40,960 to determine the end-point titer. Ten .mu.L of diluted test or control sera were placed in separate wells on the antigen slides. Slides were incubated at 37.degree. C. for 1 hr in a moist chamber, washed 3.times. for 5 min each in PBS, and then tapped gently to remove excess PBS. Fluorescein-labeled affinity-purified rabbit anti-bovine IgG diluted 1:500 in PBS was added in 10 .mu.L aliquots to each well. Slides were incubated at 37.degree. C. for 30 min, washed 3 times with PBS for 5 min each wash, tapped to remove excess PBS, cover-slipped with buffered glycerol (25% [w/v] glycerin in TRIS-HCl: pH 9.0), and examined at 200 magnification using a fluorescence microscope. The end-point titer was the last serum dilution showing distinct, whole parasite fluorescence.

Detail Description Paragraph:
[0154] Bovine Neospora isolates BPA-1 BPA-2, BPA-3, BPA-4, BPA-5 were used for DNA isolation. Parasites were harvested for DNA preparation when >80% of the CPAE cells were infected with large clusters of tachyzoites. The infected monolayer was removed from the flask by scrapping. The tachyzoites in tissue culture media were pelleted by centrifugation at room temperature, 1300.times.g for 10 minutes. The supernatant was removed and the pellet was resuspended in 10 mL sterile physiologically buffered saline (PBS:pH 7.4), passed through a 25 gauge needle three times to disrupt the CPAE cells, and then filtered through a 5 .mu.m disc filter (Gelman Sciences, Acrodisc) to remove cellular debris. The filtered material was pelleted at 1300.times.g for 10 minutes and washed in PBS (pH 7.4). The supernatant was removed and the tachyzoite pellet was stored at -70.degree. C. until used. Uninfected CPAE monolayer cells were processed by the same procedure and used as controls.

Detail Description Paragraph:
[0160] Amplification products from 3 to 5 reactions were pooled prior to purification to reduce the possibility of any nucleotide misincorporation errors by the Taq polymerase during the elongation step of the newly synthesized complement strain. The PCR amplification products were purified either by gel electroelution or by spin-columns. Two different spin-columns were used at different times. First, Magic PCR Prep DNA purification System (Promega Corp.) was used following manufacturer's directions. Briefly, the products were electrophoresed through a low temperature melting agarose (Low Melt Agarose, FMC BioPorducts,). The DNA was visualized in the gel by ethidium bromide staining and the DNA band was excised into an eppendorf tube. The agarose and DNA were heated (70.degree. C.) to melt the agarose. The DNA was separated from the agarose using columns and reagents provided in the kit. Later simpler and less time intensive methods were used by purifying the PCR products using the PCR Select II (5 Primer-3 Prime, Inc) columns which do not require electrophoresis and excision of the product in low melt agarose.

Detail Description Paragraph:
[0176] Amplification products were denatured in the gel and transferred to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, Ill.) by the Southern blotting method. DNA was cross-linked to nylon membrane using a Stratalinker UV crosslinker (Stratagene, La Jolla, Calif.). Prehybridization, preparation of the labeled internal probe, and hybridization were performed as recommended by the manufacturer for the Enhanced Chemiluminescence 3'-oligolabeling and Detection Systems (Amersham). Labeled internal probe was added to a final concentration of 10 ng/mL of hybridization solution and incubated overnight at 30.degree. C. with gentle agitation. After hybridization, the membranes were washed twice for 5 min each at room temperature in 5X SSC and 0.1% (w/v) SDS, and then washed twice for 5 min each at room temperature in 0.5X SSC and 0.1% (w/v) SDS. Membrane blocking, antibody incubations, signal generation and detection were performed as described by the manufacturer. Membranes were exposed to Kodak X-OMAT film for 3-10 min.

Detail Description Paragraph:
[0208] Rabbit antisera to the recombinant antigens, Neospora (BPA1) (Conrad, Parasitology, 106:239-249 (1993)), and Toxoplasma gondii (Conrad, Parasitology, 106:239-249 (1993)) were diluted 1:300 in TBS-Tween, 5% Blotto and incubated for 5 hours at room temperature. The secondary antibody, HRP-goat anti-rabbit (Jackson Laboratories) was diluted 1:1000 in the same buffer and incubated for 2 hours at room temperature. Blots were developed with 4-chloronaphthol and H.sub.2O.sub.2.

CLAIMS:

1. An isolated recombinant nucleic acid construct comprising a nucleic acid encoding an immunodominant Neospora antigen.

9. A method of detecting the presence of antibodies specifically immunoreactive with a bovine Neospora antigen in a biological sample, the method comprising contacting the sample with an isolated recombinant immunodominant Neospora antigen, thereby forming an antigen/antibody complex, and detecting the presence or absence of the complex.

10. The method of claim 9 wherein the Neospora antigen has the amino acid sequence shown in SEQ ID NO:10.

15. A method of detecting the presence of Neospora-specific nucleic acids in a biological sample, the method comprising contacting the sample with a oligonucleotide probe which specifically hybridizes with a target Neospora-specific polynucleotide sequence, thereby forming a hybridization complex, and detecting the presence or absence of the complex.

16. The method of claim 15, further comprising amplifying the target Neospora-specific polynucleotide sequence.

17. The method of claim 16, wherein the target Neospora-specific polynucleotide sequence is immobilized on a solid surface.

20. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an immunogenically effective amount of a bovine Neospora antigen.

21. The composition of claim 20, wherein the bovine Neospora antigen is an isolated bovine Neospora polypeptide.

23. The composition of claim 22, wherein the bovine Neospora antigen is expressed by a recombinant virus.

24. A method for protecting a bovine animal from infection by bovine Neospora, the method comprising the administration of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an immunogenically effective amount of a bovine Neospora antigen.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

12. Document ID: US 20020160441 A1

L4: Entry 12 of 21 File: PGPB Oct 31, 2002

DOCUMENT-IDENTIFIER: US 20020160441 A1
TITLE: Protein elongation factor 2 as a target for antifungal and antiparasitic agents

Summary of Invention Paragraph:
[0004] There are two EF2 genes in Saccharomyces cerevisiae, EFT1 and EFT2, and at least one of these genes is required for survival. The co-isogenic strains sS1 and sR1 were constructed by a series of genetic crosses that result in strains that are disrupted for both the EFT1 and ERG6 genes. The resultant strains are made more permeable due to the erg6 disruption (ergosterol deficient), and have either a wild-type or resistant copy of EFT2 as the only source of EF2. A known number of these yeast cells are either plated in solid medium or suspended in liquid medium and test compounds or fermentation extracts are applied with the intent of identifying samples which inhibit the growth of these yeast. The cultures are incubated at a specific temperature for a set period of time to allow for the growth of the test organisms (i.e. 30.degree. C. for 16-24 hours). Test samples of interest are those which show a differential effect on the sordarin sensitive strain(s) vs. the resistant strain(s). Those samples which are more potent against the wildtype by definition should be preventing growth via the EF2 target.

Detail Description Paragraph:
[0112] In a microfuge tube 100 .mu.l assay mixture contains: 10 .mu.g yeast S-30, 25 .mu.M GTP-.gamma.-s (0.5 .mu.l of 5 mM stock), dilutions of agent to be examined for ability to compete for binding and Buffer B to bring volume to 98 .mu.l. Vortex and incubate at room temperature 5 min. Add 2 .mu.l .sup.3H-Compound I (1:20 dilution in water). Vortex and incubate for 20-30 min.

CLAIMS:

15. The method of claim 13 wherein said parasitic disease is caused by Plasmodium, Eimeria, Isospora, Neospora, Toxoplasma, Cryptosporidium, Trypanosoma, Leishmania or Theileria.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

13. Document ID: US 20020146748 A1

L4: Entry 13 of 21 File: PGPB Oct 10, 2002

DOCUMENT-IDENTIFIER: US 20020146748 A1
TITLE: Immunological detection of neosporosis using a recombinant antigen

Abstract Paragraph:
An immunological assay method is disclosed which utilizes recombinant antigen, rNcp29, derived from an immunodominant surface antigen of Neospora caninum tachyzoites. Specifically, an ELISA method is disclosed. The method provides sensitive and specific detection of antibodies in sera of infected animals and does not exhibit cross-reaction with antisera against related parasites such as T. gondii. The ELISA method is used to screen animals for the presence of serum antibodies specific to recombinant Ncp29.

Detail Description Paragraph:
[0040] As an illustration of the above formula, using [N+]=[0.368] and 50% formamide, with GC content of 42% and an average probe size of 200 bases, the T.sub.m is 57.degree. C. The T.sub.m of a DNA duplex decreases by 1-1.5.degree. C. with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42.degree. C.

Detail Description Paragraph:
[0041] The stringency of the hybridization and wash depend primarily on the salt concentration and temperature of the solutions. In general, to maximize the rate of annealing of the probe with its target, the hybridization is usually carried out at salt and temperature conditions that are 20-25.degree. C. below the calculated T.sub.m of the of the hybrid. Wash conditions should be as stringent as possible for the degree of identity of the probe for the target. In general, wash conditions are selected to be approximately 12-20.degree. C. below the T.sub.m of the hybrid. In regards to the nucleic acids of the current invention, a moderate stringency hybridization is defined as hybridization in 6.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml denatured salmon sperm DNA at 42.degree. C., and wash in 2.times.SSC and 0.5% SDS at 55.degree. C. for 15 minutes. A high stringency hybridization is defined as hybridization in 6.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml denatured salmon sperm DNA at 42.degree. C., and wash in 1.times.SSC and 0.5% SDS at 65.degree. C. for 15 minutes. A very high stringency hybridization is defined as hybridization in 6.times.SSC, S.times.Denhardt's solution, 0.5% SDS and 100 .mu.g/ml denatured salmon sperm DNA at 42.degree. C., and wash in 0.1.times.SSC and 0.5% SDS at 65.degree. C. for 15 minutes.

Detail Description Paragraph:

[0065] The conditions for antigen binding, blocking agent addition and antibody addition, and the like, typically comprise a buffer such as phosphate-buffered saline (PBS). The buffer may contain a surfactant, for example the nonionic surfactant, Tween 80. In one embodiment, the conditions for contact include incubation at a temperature from room temperature up to about 37.degree. C., for a time adequate to allow Ab-Ag complex formation, under conditions of pH and ion concentrations that permit or promote formation of Ab-Ag complexes. In other embodiments conditions are lower temperatures and longer times, for example 4.degree. C. overnight. Following this incubation, Ab-Ag complexes are separated from the unbound antibodies or from remaining portions of a biological fluid being tested. In a preferred embodiment, the complexes bound to a solid support such as an ELISA well plate, are rinsed free of such unbound material. leaving only bound antigen and bound Ab-Ag complexes. In this embodiment, the rinse solution preferably is the same buffer used for the binding the antigen to the wells and for the addition of the blocking agent. In another embodiment, the complexes may be present in solution or may be bound to a separable support, such as a microbead, and thus separated from the remaining biological materials by conventional means, e.g., centrifugation or magnetic separation.

Detail Description Paragraph:
[0069] The exemplary method set forth below has demonstrated very good sensitivity for the detection of anti-Neospora antibodies in biological fluids, as described in greater detail in the Examples. The assay is conducted as an indirect ELISA, using a high binding capacity ELISA plate. An aliquot of a solution of purified rNcp29 (e.g., 100 .mu.l at 10 .mu.g/ml in PBS is added to each well of the plate. Following incubation for, e.g., 1 h at 37.degree. C. (or a longer time at a lower temperature), the plate is rinsed appropriately (e.g., 3 times) with PBS plus 0.05% Tween 20. The wells are then blocked for 1 h at room temperature (or for appropriately longer or shorter times at lower or higher temperatures, respectively) with 1% .gamma.-globulin-free BSA. The test sample comprises serum from animals to be tested, which is diluted 1:500 in PBS plus 0.05% Tween 20 and 0.1% BSA. Aliquots (e.g., 100 .mu.l) are added to wells of the ELISA plate and incubated 2 h at room temperature. After binding, the wells are rinsed (e.g., 3 times) with PBS plus 0.05% Tween 20. Protein G-horseradish peroxidase (HRP) conjugate diluted according to suppliers' instructions (e.g., 1:2500) in PBS plus 0.05% Tween 20 and 0.1% BSA is added (e.g., 100 .mu.l) to each well and incubated 2 h at room temperature or for appropriately shorter or longer periods depending on temperature. The plate is then rinsed well (e.g., 4 times) in PBS plus 0.05% Tween 20 and 0.1% BSA. The chromogenic substrate, o-phenylenediamine (OPD) dihydrochloride (Sigma), dissolved in 0.05 M phosphate-citrate buffer to a concentration of 0.4 mg/ml, is added (200 .mu.l) to each well. After a timed incubation (e.g., 10 min), the reaction is stopped, and the absorbance is measured at 490 nm in an ELISA plate reader. Samples tested in duplicate may be averaged and reported as mean values. The foregoing exemplary assay method detected positive titers (optical density (O.D.)>1.2 at 1:500 or greater dilution) in 13 of 13 infected cattle while 13 of 13 control animals were negative (O.D.<0.5 at 1:500). Additionally, sera from animals that were infected with T. gondii or S. cruzi did not cross-react to recombinant Ncp29 in the ELISA assay.

Detail Description Paragraph:
[0082] ELISA. Purified rNcp29 was diluted to 10 .mu.g/ml in phosphate-buffered saline (PBS) and 100 .mu.l was added per well in high-binding-capacity ELISA plate (Corning), and incubated for 1 hr at 37.degree. C. or overnight at 4.degree. C. The plate was rinsed three times with PBS containing 0.05% Tween 20 and blocked for 1 hr with PBS containing 0.05% Tween 20 and 1% .gamma.-globulin-free bovine serum albumin (BSA) (Sigma, St. Louis, Mo.). Antisera were diluted to 1:500 in PBS containing 0.05% Tween 20 and 0.1% BSA, and 100 .mu.l was added to duplicate wells of the ELISA plate, and incubated for 2 hr at room temperature (RT). The wells were rinsed three times with PBS containing 0.05% Tween and incubated for 2 hrs at RT with 100 .mu.l of Protein G-HRP conjugate (Pierce) diluted to 1:2500 in PBS

containing 0.05% Tween 20 and 0.1% BSA. Finally the plate was rinsed four times with PBS containing 0.05% Tween 20. The chromogenic substrate, o-phenylenediamine (OPD) dihydrochloride (Sigma), was dissolved in 0.05 M phosphate-citrate buffer to a concentration of 0.4 mg/ml, and 200 .mu.l of substrate was added into each well. After a 10 min incubation, the reaction was stopped with 50 .mu.l of 2 M H.sub.2SO.sub.4, and the A.sub.490 was measured in an ELISA plate reader. Samples were performed in duplicate and are reported as mean values.

CLAIMS:

1. An isolated nucleic acid molecule encoding an antigenic polypeptide comprising a mature form of an Ncp29 protein from Neospora caninum.

11. An immunological detection system for the detection of Neospora infections comprising the antigenic protein of claim 8.

12. An immunological assay method for the detection, in a biological fluid, of antibodies specific for Neospora spp. comprising the steps of: a) providing the recombinant antigen of claim 8; b) contacting the antigen with a biological fluid to be tested, under conditions wherein antibodies present in the biological fluid, with specificity for the recombinant antigen, bind to the antigen to form antibody-antigen complexes; c) separating unbound antibody, and other components of the biological fluid from the antibody-antigen complexes; d) detecting the antibody-antigen complexes; and, e) measuring the amount of detected antibody-antigen complex, the amount of antibody-antigen complex in the biological fluid being positively correlated with the amount of anti-Neospora antibody in the biological fluid.

13. The immunological assay method of claim 12 wherein the Neospora spp is Neospora caninum.

20. An ELISA assay for detecting anti-Neospora antibodies in a biological fluid, comprising the steps of: a) providing the recombinant antigen of claim 8; b) affixing the recombinant antigen to a surface to form a surface-bound antigen; c) contacting the antigen with a biological fluid to be tested, under conditions wherein antibodies present in the biological fluid that are immunologically specific for the recombinant antigen bind to the antigen to form antibody-antigen complexes that are bound to the surface; d) washing the surface to remove unbound antibody and other components of the biological fluid; d) contacting the washed antibody-antigen complexes with a Protein G-horseradish peroxidase complex; e) detecting the antibody-antigen complexes by performing an enzymatic assay of the horsradish peroxidase that forms a detectable product; and f) measuring the amount of detected antibody-antigen complexes, the amount of antibody-antigen complexes in the biological fluid being positively correlated with the amount of anti-Neospora antibodies in the biological fluid.

21. A method of assessing a disease status of an animal with respect to the disease, neosporosis, comprising the following steps: a) establishing at least one criterion of the disease status with respect to neosporosis, the criterion being based on a measure of anti-Neospora antibodies detected by the method of claim 12; b) performing the method of claim 9 on the animal to determine a titer of the anti-Neospora antibodies in an isolated biological fluid from the animal; c) correlating the determined titer with those of known positive and negative control samples; d) optionally, further correlating the determined titer and known positive and negative control samples with a standard curve; e) comparing the titer obtained with the at least one criterion for determinations of disease status; and, f) assessing the disease status of the animal according to the at least one criterion.

22. A kit for the immunological detection of anti-Neospora antibodies comprising

the recombinantly-expressed antigen of claim 8 and optionally, one or more of the following: a) serum from a known infected animal for use as a positive serum control; b) serum from an uninfected animal for use as a negative serum control; c) a blocking agent; d) one or more buffers; e) a secondary binding component selected from the group consisting of protein G, protein A, and a secondary antibody immunologically-specific for antibodies of an animal species being tested; f) reagents for a secondary detection system; g) instructions for use; h) one or more certificates of quality control; and i) controls for a calorimetric detection system.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De

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14. Document ID: US 20020146436 A1

L4: Entry 14 of 21 File: PGPB Oct 10, 2002

DOCUMENT-IDENTIFIER: US 20020146436 A1
TITLE: Neospora vaccines

Abstract Paragraph:
A Neospora caninum vaccine comprising tissue culture grown Neospora and methods of making and using said vaccines. Neospora caninum vaccines described include those containing whole Neospora tachyzoites, extracts of Neospora tachyzoites and protective antigen subunits of Neospora tachyzoites. The vaccines of this invention may be inactivated or modified live and contain adjuvants and/or stabilizers. The vaccines of this invention may be in a liquid or lyophilized form.

Summary of Invention Paragraph:
[0017] The inactivated Neospora vaccines of this invention may include stabilizers which are added before or after adjuvanting in order to maintain the antigen content over long periods of time and under adverse conditions of high or low temperatures. Stabilizers are selected from the group consisting of protease inhibitors, sugars such as sucrose and glycerol, encapsulating polymers, chelating agents such as ethylene-diaminetetracetic acid (EDTA), proteins and polypeptides such as gelatin and polyglycine and combinations thereof.

Detail Description Paragraph:
[0020] In order to determine whether Neospora caninum vaccines can produce protection against abortion in pregnant cows in a model known in the art (Ho et al., 1997), the inventors produced Neospora caninum vaccines by growing the Neospora caninum on a Vero cell line in 850 cm.sup.2 roller bottles. A vial of Working Cells of the Vero cell line was removed from liquid nitrogen storage, thawed rapidly, diluted and placed into 850 cm.sup.2 roller bottles containing 250 mL of DMEM (high glucose), hereinafter designated DMEMH, at a rate of 4.times.10.sup.7 cells per roller. The medium was supplemented with Neomycin Sulfate at 1 mL/L and Horse Serum at 5% v/v. Cells were incubated at 36 to 38.degree. C. for 5 to 7 days until the cells were between 95 and 100% confluent. The Working Cells were removed from the roller bottles by rinsing the cell sheet with Phosphate Buffered Saline (PBS) and then adding 10 mL of a Trypsin-Ethylene-diaminetetracetic acid disodium salt (EDTA) solution (2.5 g/L of Trypsin+1 g/L EDTA) to each roller bottle, agitating the bottles gently for at least 10 minutes until the cells slough from the surface and then rinsing the bottle surface with DMEMH and pooling the contents of all of the bottles. The cells from these bottles (Production Cells) were re-inoculated into new 850cm.sup.2 roller bottles at 4.5.times.10.sup.7 cells per roller bottle. The Production Cells were incubated for

24 hours at 36 to 38.degree. C. after which they were infected with freshly-passaged Neospora caninum tachyzoites of Strain BPA-1 (3.times.10.sup.8 to 4.5.times.10.sup.8/850 cm.sup.2 roller bottle). At the time of infection, the production cells were at least 50% confluent. Infected roller bottles were incubated at 36 to 38.degree. C. for 120 to 168 hours on rotating roller racks set at between 0.2 and 0.4 rpm. At that time, the cell sheet was displaying typical CPE affecting at least 80% of the cell sheet. At the end of the incubation period the Neospora fluids were harvested by pooling the contents from all of the roller bottles into a sterile vessel and a sample was removed for live Neospora titration. Acceptable harvest fluids must have a titer of at least 3.times.10.sup.5/mL. The harvest titer for the present batch was 3.times.10.sup.50/mL. The harvest fluids were frozen and thawed twice by holding the harvest fluids at -70.degree. C. and thawing them rapidly at temperatures no higher than 37.degree. C. After this treatment, the harvest fluids were inactivated for a period of 48 hours at 4.degree. C. with 0.2 M Binary Ethylenimine (BEI). After inactivation, the BEI was neutralized with 3.16 M sodium thiosulfate. The inactivated harvest fluids were concentrated by centrifugation at 3500 rpm for 15 minutes and the pellet was re-suspended in PBS to a concentration of 3.0.times.10.sup.7 based on a microscopic count. Aliquots of these inactivated and concentrated harvest fluids were adjuvanted with two different types of adjuvants in order to prepare two different vaccine formulations. One half of the inactivated and concentrated harvest fluids was adjuvanted with 10% (v/v) HAVLOGEN.RTM. while the remainder of the inactivated concentrated harvest fluid was adjuvanted with 15% (v/v) of EMULSIGEN.RTM.. HAVLOGEN.RTM. is a polymer based adjuvant containing Carbopol while EMULSIGEN.RTM. is an oil-in-water based adjuvant.

Detail Description Paragraph:
[0031] An Equine Dermal Cell Line, Master Cell Passage 11, derived from ATCC No. CCL-57 was diluted to a cell count of 2.times.10.sup.7 cells per roller bottle in a Dulbecco's Modified Eagles Medium (DMEM) containing 10% Horse Serum and inoculated into 850 cm.sup.2 roller bottles at a volume of 250 mL per roller bottle. The cells were grown to confluency after which they were infected with 2.4.times.10.sup.7 Neospora caninum tachyzoites in 14.1 mL of DMEM. Each roller bottle contained 264 mL of DMEM plus 10% Horse Serum. The neospora-infected tissue cultures were incubated at 37.degree. C. until at least 50% of the cells demonstrated CPE (approximately 7 to 9 days). Fluids were harvested and tachyzoites were centrifuged for 30 minutes at 3500 rpm in order to concentrate the harvested antigen. The pelleted concentrated Neospora caninum antigen was re-suspended in 200 mL of decanted DMEM supernatant from the centrifuged tachyzoites. This concentrated preparation containing 8.times.10.sup.6 tachyzoites per mL was frozen for 16 hours at -70.degree. C. and then thawed at room temperature. The preparation was then inactivated using 0.05 M binary ethylenimine (BEI) incubated at 4.degree. C. for 48 hours. The inactivated preparation was neutralized using 3.16 M sodium thiosulfate. Two equal aliquots of the inactivated, neutralized Neospora caninum antigen preparation were then adjuvanted with different adjuvants as in EXAMPLE 1. One half of the preparation was adjuvanted with HAVLOGEN.RTM., a Carbopol-based polymer adjuvant, by adding adjuvant to a 10% concentration (v/v). The other half of the preparation was adjuvanted with EMULSIGEN.RTM., an oil-based adjuvant, by adding adjuvant to a 15% concentration (v/v).

Detail Description Paragraph:
[0042] This experiment was conducted in order to determine the impact of Neospora caninum antigen quantity in the vaccines, and to evaluate a Neospora vaccine comprising subunit antigens. Also incorporated in this vaccine production process was the use of a "soft kill" technique which is defined as an inactivation process utilizing reduced quantities of inactivating agents and lower incubation temperatures and shorter inactivation times. For this experiment, the Neospora caninum was grown and processed in a manner similar to that described in EXAMPLE 3. The inactivation process was modified as follows. Binary ethylenimine was added to the harvested Neospora caninum to a final concentration of 0.01 M but was incubated

at room temperature for only 24 hours after which it was neutralized by addition of sodium thiosulfate to a final concentration of 0.01 M. Subunits were obtained by removing aliquots of the inactivated tachyzoite fluids, centrifuging them at 3500 rpm for 15 minutes and decanting off the supernatant fluids. The Neospora tachyzoite pellets were re-suspended in Dulbecco's Phosphate Buffered Saline (DPBS) to produce a subunit vaccine containing only the tachyzoite antigens and not the exoantigens which are excreted by the tachyzoites into the medium. A second Neospora vaccine was prepared by re-suspending the Neospora tachyzoite pellet in the supernatant fluids which had been removed and saved. Three batches of subunit DPBS re-suspended Neospora caninum were formulated to contain 1.2.times.10.sup.7, 2.4.times.10.sup.7 and 3.6.times.10.sup.7 tachyzoites per dose, respectively. Three batches of supernatant re-suspended Neospora caninum were formulated to contain equivalent numbers of tachyzoites (1.2.times.10.sup.7, 2.4.times.10.sup.7 and 3.6.times.10.sup.7) per dose. All formulations were adjuvanted with HAVLOGEN.RTM. and brought to a final 5.0 mL/dose concentration by addition of DPBS (to subunit vaccine) or supernatant fluid respectively.

CLAIMS:

1. A Neospora caninum vaccine comprising tissue culture grown Neospora.

2. The Neospora caninum vaccine according to claim 1 wherein the tissue culture grown Neospora comprises an antigen selected from the group consisting of a whole culture of Neospora tachyzoites, an inactivated tissue culture of Neospora tachyzoites, a modified live tissue culture Neospora tachyzoites, an extract from Neospora tachyzoites and one or more subunits obtained from Neospora tachyzoites.

3. The Neospora caninum vaccine according to claim 1 further comprising an inactivating agent and an adjuvant.

4. The Neospora caninum vaccine according to claim 3 wherein the inactivating agent is selected from the group consisting of formalin, beta-propiolactone, heat, binary ethylenimine, detergents and means for freezing/thawing.

5. The Neospora caninum vaccine according to claim 3 wherein the adjuvant is selected from the group consisting of polymers, oil in water, water-in-oil-in-water, lipids, aluminum hydroxide, aluminum phosphate, aluminum sulfate, immunomodulators and combinations thereof.

6. The Neospora caninum vaccine according to claim 5 wherein the polymer adjuvant is selected from the group consisting of Carbopol, HAVLOGEN.RTM. and POLYGEN.RTM..

7. The Neospora caninum vaccine according to claim 5 wherein the oil-in-water adjuvant is selected from the group consisting of EMULSIGEN.RTM. and EMULSIGEN PLUS.RTM..

8. The Neospora vaccine according to claim 1 wherein the Neospora is modified live.

9. A method for growing a Neospora caninum in a susceptible tissue culture to an amount sufficient to protect mammals against infection or abortion caused by said Neospora caninum, comprising inoculating the Neospora caninum onto said tissue culture and harvesting the replicated Neospora caninum.

10. A method of producing a Neospora vaccine comprising the steps of: a. growing Neospora caninum in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue culture grown Neospora caninum; and c. formulating said harvest into a vaccine.

11. A method of producing a Neospora vaccine comprising the steps of: a. growing

Neospora caninum in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue culture grown Neospora caninum; c. inactivating said harvested tissue culture grown Neospora caninum; and d. adjuvanting the inactivated harvested tissue culture grown Neospora caninum to produce a vaccine.

12. A method of producing a Neospora subunit vaccine comprising the steps of: a. growing Neospora caninum in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue culture grown Neospora caninum; c. extracting one or more protective antigens from the harvested tissue culture grown Neospora caninum to produce a subunit; d. inactivating the subunit(s), optionally; and e. adjuvanting the subunit(s) to produce a vaccine.

13. A method of producing a Neospora subunit vaccine comprising the steps of: a. growing Neospora caninum in a susceptible tissue culture until a cytopathic effect is produced; b. harvesting said tissue culture grown Neospora caninum; c. inactivating the Neospora caninum harvest; d. extracting one or more protective antigens from the harvested, inactivated tissue culture grown Neospora caninum to produce a subunit(s); and e. adjuvanting the subunit(s) to produce a vaccine.

14. A method of protecting mammals from disease caused by Neospora caninum comprising, administering to said mammals the vaccine according to claims 1-8.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

15. Document ID: US 20020143018 A1

L4: Entry 15 of 21 File: PGPB Oct 3, 2002

DOCUMENT-IDENTIFIER: US 20020143018 A1
TITLE: Praziquantel compounds for treating diseases due to Sarcocystis, Neospora, Toxoplasma and Isospora

Summary of Invention Paragraph:
[0027] Surprisingly, the pastes, according to the invention, are effective when used in treating the parasites. More specifically, it is surprising that the pastes of the present invention are deliverable in a neurologically-effective dose. They are effective in delivering the praziquantel to cross the blood-brain or placenta barrier and attack the parasites that have already invaded the brain or infected the fetus of a pregnant animal. As a matter of convenience, there is provided herein a description of a specific embodiment of the pastes preferred herein and how it is prepared. A preferred paste, according to the present invention contains a micronized suspension of the praziquantel, propylene glycol, a thickening agent such as Carbopol, preservatives such as Methylparaben and Propylparaben, and water. The paste can be made by combining water, typically, purified water and Propylene Glycol, heating the combination to about 70.degree. C., and adding the preservatives, at this temperature. The resulting mixture is cooled to room temperature after which Carbopol, preferably in the form of Carbopol 974P or 934P, is added. Finally the praziquantel is added. After complete mixing, the pH is adjusted to approximately 6.0 with sodium hydroxide. The most preferable paste includes 15% w/w Praziquantel, 20% w/w Propylene Glycol, 0.5% w/w Carbopol 974P, 0.14% w/w Methylparaben, 0,02% w/w Propylparaben, 0.1 % w/w sodium hydroxide with the remainder being purified water. Sweeteners including dextrose, sucrose, lactose, fructose, sorbitol, xylitol, artificial sweeteners and molasses may be added to improve palatability. Additionally, yeast or liver flavoring may be added for the same purpose.

CLAIMS:

3. The composition of claim 2 wherein the coccidia is a member of the group consisting of Sarcocystis spp, Neospora spp, Toxoplasma spp and Isospora spp.

4. The composition of claim 3 wherein the Sarcocystis spp is Sarcocystis neurona, the Neospora spp is Neospora caninum or Neospora hugesi, the Toxoplasma spp is Toxoplasma gondii and the Isospora spp is Isospora suis.

6. The composition of claim 4 wherein the Neospora caninum is the causative agent of bovine or canine Neosporosis.

7. The composition of claim 4 wherein the Neospora hugesi is the causative agent of Equine Protozoal Myeloencephalyitis.

12. The method of claim 11 wherein the coccidia is a member of the group consisting of Sarcocystis spp, Neospora spp, Toxoplasma spp and Isospora spp.

13. The method of claim 12 wherein the Sarcocystis spp is Sarcocystis neurona, the Neospora spp is Neospora caninum or Neospora hugesi, the Toxoplasma spp is Toxoplasma gondii and the Isospora spp is Isospora suis.

15. The method of claim 12 wherein the Neospora caninum is the causative agent of bovine or canine Neosporosis.

16. The method of claim 12 wherein the Neospora hugesi is the causative agent of Equine Protozoal Myeloencephalyitis.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

16. Document ID: US 20020131979 A1

L4: Entry 16 of 21 File: PGPB Sep 19, 2002

DOCUMENT-IDENTIFIER: US 20020131979 A1
TITLE: Adjuvanted vaccine which is substantially free of non-host albumin

Detail Description Paragraph:
[0062] The inactivated antigen can also be concentrated or pooled with other harvested antigen prior to adjuvanting. The amount of concentration would be such that the average amount of antigen or Relative Potency (RP) value meets or exceeds the minimum acceptable value for a vaccine. The inactivated antigen may be concentrated up to 100 fold, if necessary, by ultrafiltration with a molecular weight cut-off which will suitably maintain the antigen and allow contaminants to pass through and be discarded or by differential centrifugation. After inactivation, the antigen value must be above the acceptable minimum level or RP. Then it is stored at temperatures from -70.degree. C. to +10.degree. C. until it is mixed or microfluidized with an adjuvant.

Detail Description Paragraph:
[0071] CRFK cells (Crandell Feline Kidney) persistently infected with FeLV were grown to 95% confluency as follows. The cells were grown in 850 cm2 roller bottles

incubated with rotation at 37.degree. C. Employed as the growth medium was Dulbecco's Minimal Essential Medium with high glucose levels (DMEM-Hi) containing 10% fetal bovine serum and 30 ug/ml neomycin. After the cells reached confluency, the media was changed to maintenance media (DMEM-Hi media containing 5% fetal bovine serum). After four days this media was decanted and viral fluids were harvested. Cells were re-fed with maintenance media and viral fluids were collected every three to four days for a total of seven harvests. Decanted viral fluids from each harvest were tested for sterility, aliquoted into sterile plastic containers and stored frozen at -70.degree. C. Upon satisfactory sterility testing, viral fluids were thawed at room temperature and pooled into a single sterile receiving vessel. Viral fluids were clarified through a 3 micron polypropylene filter to remove cell debris and then concentrated 10-fold using a 30,000 dalton molecular weight cut-off tangential flow ultrafiltration device. Fluids were then washed in 50 Mm Na.sub.2HPO.sub.4 to a 9-fold final concentration factor. The pooled concentrate had a total protein content of 16.59 mg/mL.

Detail Description Paragraph:
[0078] CRFK cells persistently infected with FeLV were grown to 95% confluency in DMEM-Hi containing 10% fetal bovine serum and 30 ug/mL neomycin using 850 cm.sup.2 roller bottles incubated with rotation at 37.degree. C. as in EXAMPLE 2A. After the cells reached confluency, the media was changed to maintenance media (DMEM-Hi media containing 5% fetal bovine serum). After four days, this media was decanted and viral fluids were harvested. Cells were refed with maintenance media and viral fluids were collected every three to four days for a total of seven harvests. Decanted viral fluids from each harvest were tested for sterility. All harvest fluids were found to be sterile. Viral fluids from each harvest were aliquoted into sterile plastic containers and stored frozen at -70.degree. C. Upon satisfactory sterility testing, viral fluids were thawed at room temperature and pooled into a single sterile receiving vessel. The total protein content of this pooled FeLV was 2.5 mg/mL. Viral fluids were clarified through a 5 micron and a 1 micron polypropylene filter to remove cell debris.

CLAIMS:

6. The serum-based vaccine of claim 2 wherein the parasite antigen is selected from the group consisting of Toxoplasma spp., Dirofilaria spp., Cryptosporidium spp., Coccidia spp., Babesia spp., Neospora spp., subunits therefrom and combinations thereof.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

17. Document ID: US 20020102273 A1

L4: Entry 17 of 21 File: PGPB Aug 1, 2002

DOCUMENT-IDENTIFIER: US 20020102273 A1
TITLE: USE OF ALPHAVIRUS EXPRESSION VECTORS TO PRODUCE PARASITE ANITGENS

Detail Description Paragraph:
[0067] Sindbis virus gene expression, which occurs in the cytoplasm of the cell, is quite efficient, rapid, and can be modulated. For example, Xiong et al., ibid., reported the production of up to 1.times.10.sup.8 molecules of chloramphenicol acetyltransferase (CAT) per cell transfected with Sindbis virus expression vectors

operatively linked to the CAT gene, when the cell was cultured for about 20 hr. Xiong et al. also reported that use of a replication temperature sensitive Sindbis virus vector led to modulated expression of CAT.

Detail Description Paragraph:
[0082] After transfection, transfected cells are cultured in an effective medium, using techniques such as those described in Xiong et al., ibid. As used herein, an effective medium refers to any medium in which the transfected cells can produce recombinant virus particle vaccines. An effective medium is typically an aqueous medium comprising assimilable carbohydrate, nitrogen and phosphate sources, as well as appropriate salts, minerals, metals and other nutrients, such as vitamins, growth factors and hormones. culturing is carried out at a temperature, pH and oxygen content appropriate for the transfected cell. Such culturing conditions are well within the expertise of one of ordinary skill in the art. Examples of preferred effective media are included in the Examples section.

Detail Description Paragraph:
[0110] In order to produce protective compounds of the present invention, a recombinant cell, produced as described above, is cultured in an effective medium, using techniques such as those described in Xiong et al., ibid. As used herein, an effective medium refers to any medium in which the transfected cells can produce protective compounds of the present invention. An effective medium is typically an aqueous medium comprising assimilable carbohydrate, nitrogen and phosphate sources, as well as appropriate salts, minerals, metals and other nutrients, such as vitamins, growth factors and other hormones. The medium may comprise complex nutrients or may be a defined medium. Recombinant cells of the present invention can be cultured in conventional fermentation bioreactors, which include, but are not limited to, batch, fed-batch, cell recycle and continuous fermentors. Culturing can also be conducted in shake flasks, test tubes, microtiter dishes and petri plates. Culturing is carried out at a temperature, pH and oxygen content appropriate for the recombinant cell. Such culturing conditions are well within the expertise of one of ordinary skill in the art. Examples of preferred effective media and culturing conditions are included in the Examples section.

Detail Description Paragraph:
[0158] In one experiment, recombinant virus particle vaccine VPV SV2:nP30.1008 is produced by co-transfecting baby hamster kidney (BHK) cells with recombinant molecule SV2:nP30.1008 and packaging vector PV1 using electroporation in a manner similar to that described by Liljestrom et al., ibid. Briefly, BHK cells are grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells are trypsinized, washed once with Minimal Essential Medium (also called MEM; available from Life Technologies Inc., Gaithersburg, MD) containing 10% fetal calf serum, washed once with ice cold phosphate buffered saline (8 g NaCl, 0.2 g KCl, 1.44 g Na.sub.2HPO.sub.4, 0.24 g KH2PO4 per liter of water, the pH of which is adjusted to about pH 7.4; also called PBS) and resuspended in PBS at about 1.times.10.sup.7 cells per ml. About 0.5 ml of cells and about 5-10 .mu.g (in about 10-50 microliters (.mu.l)) total of SV2:nP30.1008 and PV1 (at a mole/mole ratio of about 1:1) are mixed in a 0.2 centimeter (cm) cuvette suitable for use in Bio-Rad's Gene Pulser Apparatus (both available from Bio-Rad Laboratories, Richmond, Calif.). The RNA either may be used directly from the in vitro transcription reaction mixture (as described in Example 2 for the recombinant molecule and in Example 3 for the packaging vector) or may be diluted with transcription buffer containing 5 millimolar (mM) dithiothreitol and 1 unit of RNasin per ml. Electroporation is conducted at room temperature by two consecutive pulses at 1.5 kilovolts (KV) and 35 microfarads (.mu.F), using the Gene Pulser Apparatus with the pulse controller unit set at maximum resistance. After electroporation, the cells are diluted about 1:20 in complete BHK cell medium and transferred to tissue culture plates. The cells are then cultured for about 24 to about 36 hours at about 37.degree. C. and about 5% carbon dioxide in about 5 ml of MEM with 10% fetal calf serum.

Detail Description Paragraph:
[0168] A recombinant cell capable of expressing the GST-P30.257 fusion protein was produced by transfecting SV3:nGST-nP30.771 into baby hamster kidney (BHK) cells using electroporation in a manner similar to that described by Liljestrom et al., ibid. Briefly, BHK cells were grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells were trypsinized, washed once with Minimal Essential Medium (also called MEM; available from Life Technologies Inc., Gaithersburg, Md.) containing 10% fetal calf serum, washed once with ice cold phosphate buffered saline (8 g NaCl, 0.2 g KCl, 1.44 g Na.sub.2HPO.sub.4, 0.24 g KH.sub.2PO.sub.4 per liter of water, the pH of which is adjusted to about pH 7.4; also called PBS) and resuspended in PBS at about 1.times.10.sup.7 cells per ml. About 0.5 ml of cells and about 5-10 .mu.g (in about 10-50 .mu.l) of SV3:nGST-nP30.771 were mixed in a 0.2 centimeter (cm) cuvette suitable for use in Bio-Rad's Gene Pulser Apparatus (both available from Bio-Rad Laboratories, Richmond, Calif.). The RNA was either used directly from the in vitro transcription reaction mixture (as described in Example 2) or was diluted with transcription buffer containing 5 millimolar (MM) dithiothreitol and 1 unit of RNasin per ml. Electroporation was conducted at room temperature by two consecutive pulses at 1.5 kilovolts (KV) and 35 microfarads (.mu.F), using the Gene Pulser Apparatus with the pulse controller unit set at maximum resistance. After electroporation, the cells were diluted about 1:20 in complete BHK cell medium and transferred to tissue culture plates.

Detail Description Paragraph:
[0182] A recombinant cell capable of expressing Di22.RA was produced by transfecting SV3:nDi22.RA into BHK cells using lipofection in a manner similar to that described by Felgner et al., pp. 7413-7417, 1987, Proc. Natl. Acad. Sci. USA, Vol. 84. Briefly, BHK cells were grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells were washed with PBS and incubated for about 10 minutes at about room temperature with a mixture of from about 0.25 to about 1.0 .mu.g of SV3:nDi22.RA and about 20 .mu.g of Lipofectin (available from Life Technologies Inc., Gaithersburg, Md.) in about 0.4 ml PBS. The mixtures was then removed and the cells washed two times with PBS and incubated with about 5 ml of MEM containing 10% fetal calf serum for about 24 to about 36 hours at about 37.degree. C. in order to produce recombinant virus. BHK cells were infected by the recombinant virus and incubated for about 12 to about 16 hours at about 37.degree. C. to produce Di22.RA.

Detail Description Paragraph:
[0185] Packaging-competent recombinant molecule SV3:nDi22.RA, produced as described in Example 9, was packaged into a viral coat as follows. SV3:nDi22.RA was transfected into BHK cells using lipofection in a manner similar to that described by Felgner et al., pp. 7413-7417, 1987, Proc. Natl. Acad. Sci. U.S.A., Vol. 84. Briefly, BHK cells were grown in 60 mm tissue culture plates to a monolayer confluency of about 90%. The cells were washed with PBS and incubated for about 10 minutes at about room temperature with a mixture of from about 0.25 to about 1.0 .mu.g of SV3:nDi22.RA and about 20 .mu.g of Lipofectin (available from Life Technologies Inc., Gaithersburg, Md.) in about 0.4 ml PBS. The mixtures was then removed and the cells washed two times with PBS and incubated with about 5 ml of MEM containing 10% fetal calf serum for about 24 to about 36 hours at about 37.degree. C. in order to produce recombinant virus RV SV3:nDi22.RA, also known as HJA.

CLAIMS:

5. The vaccine of claim 1, wherein said disease is caused by an infectious agent selected from the group consisting of the genera Toxoplasma, Dirofilaria, Acanthocheilonema, Babesia, Brugia, Candida, Cryptococcus, Cryptosporidium, Dipetalonema, Eimeria, Encephalitozoon, Hepatozoon, Histoplasma, Isospora, Loa, Microsporidia, Neospora, Nosema, Onchocerca, Parafilaria, Plasmodium, Pneumocystis, Rochalimaea, Setaria, Stephanofilaria, Theileria and Wuchereria.

42. The recombinant molecule of claim 40, wherein said parasite is selected from the group consisting of Toxoplasma, Dirofilaria, Acanthocheilonema, Babesia, Brugia, Candida, Cryptococcus, Cryptosporidium, Dipetalonema, Eimeria, Encephalitozoon, Hepatozoon, Histoplasma, Isospora, Loa, Microsporidia, Neospora, Nosema, Onchocerca, Parafilaria, Plasmodium, Pneumocystis, Rochalimaea, Setaria, Stephanofilaria, Theileria and Wuchereria.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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18. Document ID: US 20020058046 A1

L4: Entry 18 of 21 File: PGPB May 16, 2002

DOCUMENT-IDENTIFIER: US 20020058046 A1
TITLE: Neospora vaccine

Abstract Paragraph:
The present invention provides an homogenate prepared from cells of Neospora, and vaccines against neosporosis prepared therefrom which are useful in the prevention of clinical disease and abortion in mammals.

Detail Description Paragraph:
[0036] Cells which may be used to produce the cell homogenate of the invention are preferably tachyzoites, but may alternatively be bradyzoites or oocysts, or some combination thereof. In addition, cells for use in the present invention may either be viable cells or cells which have previously been inactivated, e.g., by treatment with a chemical inactivating agents such as formaldehyde or glutaraldehyde, among others, or by treatment with radiation, or by exposure to extreme pH or temperature, or some combination thereof.

Detail Description Paragraph:
[0069] The tachyzoite preparation was frozen (-20.degree. C.) and thawed (room temperature) three times, and then sonicated (Branson Sonifer 250, Branson Inc.) at a constant output (4 minutes/cycle) for three cycles on ice. The resulting homogenate was designated as a Neospora antigen (NSA) preparation. The protein concentration of the NSA preparation was determined using a commercial assay (Pierce BCA). NSA preparation aliquots were prepared and stored at -20.degree. C. or -70.degree. C. until further use in a vaccine and for in vitro assays (e.g., Western blot, cell proliferation). The NSA preparation did not contain any viable tachyzoites, as determined by lack of in vitro growth in MARC-145 cells and the inability to kill immunodeficient, nude mice.

Detail Description Paragraph:
[0074] In the first part of the study, 8 week old female BALB/c mice (n=10/group) were immunized at day 0 and again at day 21 with either the SEAM62 adjuvant alone (control) or the NSA preparation plus the SEAM62 adjuvant (vaccine). Fifteen days after the last immunization, individual serum samples were randomly collected from 3 mice per group and stored at -20.degree. C. for analysis of parasite-specific antibodies by Western blot (FIG. 1). The post-immunization Western blot analysis was conducted as follows. The NSA preparation was fractionated alongside molecular weight markers (Novex, San Diego, Calif.) under standard, nonreducing conditions by preparative gel electrophoresis (SDS-PAGE) using 12% sodium dodecyl sulfate-

polyacrylamide precast gels (Novex). Following electrophoresis, separated proteins were transferred to PVDF membrane (Millipore, Bedford, Mass.), which was then rinsed in wash buffer (phosphate buffered saline (pH 7.5)/0.5% Tween 20 detergent), air-dried, and individual membrane strips cut (approx. 8 .mu.g NSA protein/strip). Strips were incubated overnight at room temp. in blocking buffer (wash buffer containing 5% skim milk). Following two brief washes, strips were incubated for 1 hr at room temp. with primary antiserum samples (1:200 dilution in wash buffer) obtained at 15 days after the last immunization from either 3 individual adjuvant control mice (FIG. 1, lanes 1-3) or 3 vaccinated mice (FIG. 1, lanes 4-6). Following two rinses in wash buffer. strips were incubated with alkaline-phosphatase conjugated goat anti-mouse IgG (Kirkegaard & Perry) (1:10,000 dilution in wash buffer) for 1 hr at room temp, rinsed twice in wash buffer, and immunoreactive proteins detected using the chromogenic substrate BCIP/NBT (5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium) (Kirkegaard & Perry).

Detail Description Paragraph:
[0078] Pre- and post-challenge immunofluorescence antibody (IFA) titer assays were conducted as follows. Viable NC-1 tachyzoites (5.times.10.sup.4) were added to each well of a 96-well flat bottom plate. Wells were air-dried, and plates were stored at -20.degree. C. until used. Serum test samples collected on day 21 post-immunization (day 0 challenge) and day 21 post-challenge were tested for IFA titers. Starting at an initial 1:50 serum dilution, serial twofold dilutions were added to wells and incubated for 30 min at room temperature. Following two washes in carbonate rinse buffer, wells were incubated with (Fab).sub.2 fluorescein isothiocyanate-conjugated anti-mouse IgG+IgM (Southern Biotechnology, Birmingham., Ala.). The plates were washed and 50 .mu.l of 50% glycerol diluted in rinse buffer was added to each well. Plates were stored at 4.degree. C. until viewed under a fluorescence microscope equipped with a filter for emission at 510 nm. Antibody titers are based on the highest dilution of immune serum producing detectable fluorescence.

CLAIMS:

1. An homogenate prepared from cells of Neospora which is capable of inducing a protective response against neosporosis in a mammal.

2. The homogenate of claim 1, wherein the species of Neospora from which the homogenate is prepared is N. caninum.

4. The homogenate of claim 3, wherein the species of Neospora from which the homogenate is prepared is N. caninum.

7. A vaccine to protect a mammal against neosporosis, comprising an immunologically effective amount of an homogenate prepared from cells of Neospora, which homogenate is capable of inducing a protective response against neosporosis in a mammal, and a veterinarily acceptable carrier.

8. The vaccine of claim 7, wherein the species of Neospora from which the homogenate is prepared is N. caninum.

10. The vaccine of claim 9, wherein the species of Neospora from which the homogenate of the vaccine is prepared is N. caninum.

18. The vaccine of claim 7, wherein the Neospora cells from which the homogenate is prepared have been modified to delete the expression of one or more antigenic components normally associated with Neospora cells or a homogenate prepared therefrom.

19. A method for preparing a vaccine that protects a mammal against neosporosis, comprising homogenizing cells from Neospora to produce an homogenate capable of

inducing a protective response against neosporosis in a mammal, and combining an immunologically effective amount of the homogenate with a veterinarily acceptable carrier.

20. The method of claim 19, wherein the species of Neospora from which the homogenate is prepared is N. caninum.

22. The method of claim 21, wherein the species of Neospora from which the homogenate of the vaccine is prepared is N. caninum.

29. A method for protecting a mammal against neosporosis, comprising administering to the mammal a vaccine comprising an immunologically effective amount of an homogenate prepared from cells of Neospora, which homogenate is capable of inducing a protective response against neosporosis in a mammal, and a veterinarily acceptable carrier.

30. The method of claim 29, wherein the species of Neospora from which the homogenate is prepared is N. caninum.

32. The method of claim 31, wherein the species of Neospora from which the homogenate of the vaccine is prepared is N. caninum.

39. A combination vaccine for protecting a mammal against neosporosis and, optionally, one or more other diseases or pathological conditions that can afflict the mammal, which combination vaccine comprises an immunologically effective amount of a first composition comprising an homogenate prepared from cells of Neospora, which homogenate is capable of inducing a protective response against neosporosis in a mammal; an immunologically effective amount of a second composition capable of inducing a protective response against a disease or pathological condition that can afflict the mammal; and a veterinarily acceptable carrier.

40. The combination vaccine of claim 39, wherein the species of Neospora from which the homogenate of the first composition is prepared is N. caninum.

42. The combination vaccine of claim 41, wherein the species of Neospora from which the homogenate of the first composition is prepared is N. caninum.

45. The combination vaccine of claim 39, wherein the second composition is capable of inducing in the mammal a protective response against a pathogen selected from the group consisting of bovine herpes virus, bovine respiratory syncitial virus, bovine viral diarrhea virus, parainfluenza virus types I, II, or III, Leptospira spp., Campylobacter spp., Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma spp., Klebsiella spp., Salmonella spp., rotavirus, coronavirus, rabies, Pasteurella haemolytica, Pasteurelia multocida, Clostridia spp., Tetanus toxoid, E. coli, Cryptosporidium spp., Eimeria spp. and Neospora spp.

46. A kit for vaccinating a mammal against neosporosis, comprising a first container having an immunologically effective amount of an homogenate prepared from cells of Neospora, which homogenate is capable of inducing a protective response against neosporosis in a mammal, and a second container having a veterinarily acceptable carrier or diluent.

47. An antibody specific to an antigenic component present in an homogenate of Neospora cells.

19. Document ID: US 20020044952 A1

L4: Entry 19 of 21 File: PGPB Apr 18, 2002

DOCUMENT-IDENTIFIER: US 20020044952 A1
TITLE: Attenuated live neospora vaccine

Abstract Paragraph:
The present invention provides attenuated live cultures of the pathogenic protozoan parasite, Neospora, and live vaccines against neosporosis prepared therefrom which are useful in the prevention of clinical disease and abortion in mammals.

Summary of Invention Paragraph:
[0021] High serial passage may be carried out by repeated in vitro passaging of cells of a pathogenic strain of Neospora in susceptible host cells until sufficient attenuation occurs. Passaging may be conducted under specific environmental conditions to select for attenuated cells. For example, passaging may be conducted at a temperature below that of the body temperature of the intended mammalian vaccinate to select for temperature-sensitive strains of Neospora that will not grow, or that will only grow at a reduced rate, when administered in a vaccine to the mammal.

Summary of Invention Paragraph:
[0034] After the attenuation step, cells that exhibit one or more indicators of attenuated pathogenicity are selected from the culture and clonally propagated after limiting dilution. Examples of such indicators include, but are not limited to, appearance of a novel temperature-sensitivity or a novel auxotrophy in vitro, or a reduction in a virulence attribute such as infectivity or severity or rate of progression of one or more symptoms or conditions in a mammal after administration of cells of the strain as compared to infection with the parent strain, among others. A particular, non-limiting example of a temperature-sensitivity that is useful in practicing the invention is one in which cells of the attenuated strain will grow at 32.degree. C., but not at 37.degree. C. Such a temperature-sensitive strain will cause the lysis of infected host cells at 32.degree. C., resulting in the appearance of lesions or plaques in a host cell monolayer. When grown at 37.degree. C., the attenuated strain will not replicate sufficiently and will thus fail to produce plaques in host cell monolayers.

Detail Description Paragraph:
Establishment of Temperature-Sensitive Strains of N. caninum and Analysis of Pathogenicity in BALB/c Mice

Detail Description Paragraph:
[0052] The objective of this study was to establish temperature-sensitive strains of N. caninum (NCTS), and to test the pathogenicity of these strains by analyzing serum antibody response, tissue cyst and brain lesion production, and the development of clinical symptoms in BALB/c mice, which are known to be highly susceptible to neosporosis.

Detail Description Paragraph:
[0054] Tachyzoites of the NC-1 strain were mutagenized by exposure to 0.5 .mu.M N-methyl-N'-nitro-N-nitrosoguanidine (Sigma) in growth medium for 24 hr, and then grown at 32.5.degree. C. for 3 mos in Hs68 cells in maintenance medium, after which tachyzoites were cloned by limiting dilution. Twelve clones were initially isolated. Three clones, designated as NCTS4, NCTS-8, and NCTS-12 (NCTS=N. caninum,

temperature-sensitive), were selected for further study after being maintained in Hs68 cells in continuous culture at 32.5.degree. C. for >8 mos in maintenance medium.

Detail Description Paragraph:
[0084] A first objective of this study was to determine if vaccination with a live, temperature-sensitive strain of N. caninum can provide protection against disease caused by subsequent challenge with a pathogenic strain, e.g., NC-1, of N. caninum. A second objective of this study was to determine the level of protection provided by vaccination of BALB/c mice with killed (frozen) NCTS8 tachyzoites that were subsequently challenged with the NC-1 strain of N. caninum.

Detail Description Paragraph:
[0113] Does in groups A-D were challenged with 4.times.10.sup.6 tachyzoites of the NC-1 strain of N. caninum in serum-free maintenance medium (0.45 ml) administered by jugular i.v. The does were then monitored by ultrasound, by temperature taken daily for 7 days post-challenge, and by visual observation twice daily, and were bled once per week post-challenge.

CLAIMS:

1. A culture of cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects a mammal against neosporosis when administered as a live vaccine.

2. The culture of claim 1, the cells of which are temperature-sensitive.

6. A vaccine to protect a mammal against neosporosis, comprising an immunologically effective amount of live cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects the mammal against neosporosis when administered as a live vaccine, and a veterinarily acceptable carrier.

7. The vaccine of claim 6, wherein the attenuated cells are temperature-sensitive.

13. A method for preparing a culture of attenuated cells of a species of Neospora for use in a vaccine that protects a mammal against neosporosis, comprising modifying cells from a pathogenic parent strain of a species of Neospora; selecting and clonally propagating one or more modified cells that exhibit attenuated pathogenicity compared to cells of the parent strain; and selecting and clonally propagating one or more attenuated cells which are capable of triggering an immune response that protects the mammal against neosporosis when administered in a live vaccine.

14. The method of claim 13, in which the cells of the attenuated culture are temperature-sensitive.

17. A method for preparing a vaccine to protect a mammal against neosporosis, comprising modifying cells from a pathogenic parent strain of a species of Neospora; selecting and clonally propagating those modified cells that exhibit attenuated pathogenicity compared to cells of the parent strain but which are capable of triggering an immune response in the mammal that protects against neosporosis when administered in a live vaccine; and combining an immunologically effective amount of the attenuated cells with a veterinarily acceptable carrier in a form suitable for administration as a live vaccine to the mammal.

18. The method of claim 17, wherein the attenuated cells are temperature-sensitive.

24. A method of vaccinating a mammal against neosporosis, comprising administering to the mammal an immunologically effective amount of a vaccine comprising live cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects the mammal against neosporosis when administered as a live vaccine, and a veterinarily acceptable carrier.

25. The method of claim 24, wherein the attenuated cells are temperature-sensitive.

32. A combination vaccine, comprising an immunologically effective amount of live cells of a strain derived from a pathogenic parent strain of a species of Neospora, which cells exhibit attenuated pathogenicity compared to those of the parent strain but which are capable of triggering an immune response that protects the mammal against neosporosis when administered as a live vaccine; one or more other antigens that trigger an immune response that protects the mammal against a disease or a pathological condition; and a veterinarily acceptable carrier.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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20. Document ID: JP 10167983 A

L4: Entry 20 of 21 File: JPAB Jun 23, 1998

PUB-NO: JP410167983A
DOCUMENT-IDENTIFIER: JP 10167983 A
TITLE: ATTENUATED LIVE NEOSPORA VACCINE

PUBN-DATE: June 23, 1998

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INT-CL (IPC): A61 K 39/00; A61 K 35/68; A61 K 39/002; A61 K 39/39; C12 N 1/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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21. Document ID: WO 9808970 A1

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PUB-NO: WO009808970A1
DOCUMENT-IDENTIFIER: WO 9808970 A1
TITLE: DETECTING ITS1 IN TOXOPLASMA GONDII AND NEOSPORA CANINUM USING PCR

PUBN-DATE: March 5, 1998

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INT-CL (IPC): C12 Q 1/68
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Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw D

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